FERMENTED FOOD PRODUCTS

Edited by

A. Sankaranarayanan

N. Amaresan

D. Dhanasekaran

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Fermented Food Products

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Edited by

A. Sankaranarayanan
(Uka Tarsadia University)

N. Amaresan
(Uka Tarsadia University)

D. Dhanasekaran
(Bharathidasan University)

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Foreword

The widely used fermented food products are enriched with beneficial probiotic microorganisms which provide a wide range of health benefits from better digestion to stronger immunity. Their significant role in the diet of developed, developing, and underdeveloped countries human population is commendable. The fermentation process also enhances the nutritive value of the food and minimizes the toxicity of the food material. In addition, the fermented food products also contribute to the cultural identity, local economy, and gastronomical delight.

Apart from the widely popular fermented food products, some of the traditional fermented food products have also been included in this book. Currently, there is a renewed interest in traditional fermented foods, mainly driven by the purported health benefits of fermented foods, both as vehicles of probiotic organisms and health-promoting metabolites.

The book comprises a total of 23 chapters listed under eight sections which deal with the different views of fermented food products from Global diversity to molecular tools involved. Furthermore, the book emphasizes specific ethnic fermented foods and beverages made from diverse sources *viz.* animal, plant and milk, etc. and used in different countries. The book caters the different geographic regions of the world with respect to the fermented food products, consumption as well as their therapeutic uses. I am sure that the readers will attain updated and recent information about different kinds of fermented food products.

I congratulate the editorial team and contributors of this book and appeal to the readers, including young researchers, scientists, and academicians to make use of this interesting book.

Myunghee Kim

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Preface

Fermentation is a traditional process which is generally used to preserve the food from spoilage in the earlier days. It was primarily developed for the stabilization of perishable agricultural produce. Notwithstanding, the technology has evolved beyond food preservation into a tool for creating desirable organoleptic, nutritional and functional attributes in food products. Fermented food products still make up a significant portion of the diet in developing countries. Now a new dimensional approach leads to the enhancement of nutritional properties, inhibition of spoilage and contaminant organisms, and conversion of non-nutritive foods into nutritive ones by fermentation process. Moreover, the concept of developing therapeutic food products is appealing and growing nowadays. Apart from this, the existing reports denoted the degradation of toxic and non-nutritive factors, and this process played a pivotal role in food technology by means of fermented food products since they act as a bio-preservative by producing organic acids, anti-oxidants, and antimicrobial compounds.

This book is intended to provide a comprehensive overview about the different dimensions of fermented food products used in the world. These aspects include the global overview about the fermented food products such as plant-, animal-, and dairy-based fermented food products, as well as nutritive values of fermented beverages. The common fermented foods include kefir, kombucha, sauerkraut, pickles, miso, tempeh, natto, kimchi, curd, cheese, yogurt, beer, wine, vinegar, idli, dosa, porridge, and dhokla. The different chapters of this book mainly cover the traditional fermented food products of various geographical locations of world, application of molecular tools in fermented food products and their probable nutritional and therapeutic benefits. Since a lot of traditional fermentation-based food products are still not reaching to the scientific community and the population worldwide, this book is a scientific approach towards educating and spreading the awareness of such beneficial fermented foods with interesting information.

Furthermore, the recent scientific reports revealed that the exact mechanism of microorganisms is still unknown in some traditional fermented food products, hence, the book also provides the future insights for research in these traditional foods. In this book, a total of 23 chapters are placed under seven different sections, along with a special section allotted for traditional fermented food products of multifarious countries.

This book can be used as a source of information in different fields like microbiology, biotechnology, food technology, food science, food engineering, food chemistry as well as nutrition and health sciences. We the editors strongly believe that the book will be helpful to readers in all aspects including academic and research and it will certainly drive the future research in traditional fermented food products in order to reveal the precise role of microorganisms in food fermentation.

A. SANKARANARAYANAN
N. AMARESAN
D. DHANASEKARAN

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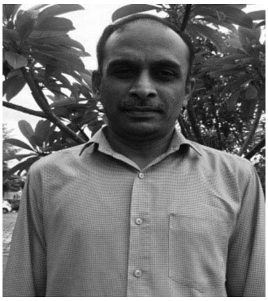
About the Editors



Dr. Sankaranarayanan is associated with C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gujarat, India, from 2015 onwards. He has experience in the fields of Antimicrobial activity of herbal and nano particles against MDR pathogens and fermented food products. His current research focus is on microbes in fermented food products and removal of bacteria from food by dielectrophoresis. He has published six chapters in various books and 50 research articles in International and National journals of repute, has guided 4 Ph.D. and 16 M.Phil. scholars and operated five minor funded projects. From 2002 to 2015, he worked as an Assistant Professor & Head, Department of Microbiology, K.S.R. College of Arts & Science, Tiruchengode, Tamil Nadu. He has been awarded with the Indian Academy of Sciences (IASc), National Academy of Sciences (NAS), and The National Academy of Sciences (TNAS)–sponsored summer research fellowship for young teachers consecutively for three years. His name is included as a Mentor in DST-Mentors/Resource persons for summer/winter camps and other INSPIRE initiatives, Department of Science & Technology, Govt. of India, New Delhi. He is a Grant reviewer in British Society of Antimicrobial Chemotherapy (BSAC), UK. He has involved himself in the organization of various National/International seminars/symposia. He is actively involved as an Editor/Editorial board member in journals and reviewers in various International/National reputed journals and acted as an external examiner to adjudicate the Ph.D. thesis of various universities in India.

Dr. N. Amaresan is an Assistant Professor at C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gujarat, India. He is basically a microbiologist, and has obtained his Ph.D. degree on endophytic PGP bacteria from Bharathidasan University, Tamil Nadu. Dr. Amaresan has over 13 years of experience in teaching & research and made several original and novel discoveries, especially in various allied fields of microbiology mainly plant-microbe interactions, bioremediation, plant pathology and others. For his original discoveries on agriculturally important microorganisms, he has been awarded young scientist awards by the Association of Microbiologists of India and National Academy of Biological Sciences. He has also been awarded visiting scientist fellowship from the National Academy of India to learn advanced techniques. He has handled two institute-funded and four externally funded projects from DST, GEMI, DBT, and so on. He has published more than 50 research articles and books of national and international reputation. He has also deposited over 350 bacterial 16S rDNA and fungal ITS rDNA sequences in the GenBank (NCBI, EMBL & DDBJ) and also has preserved over 150 microbial germplasm in various culture collection centers of India.

Dr. Dharumadurai Dhanasekaran is an Assistant Professor at the Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India. He has experience in the fields of actinobacteriology, mycology, and phycology. His current research focus is on actinobacteria, microalgae, fungi, and mushroom for animal and human health improvement. He has been awarded UGC-Raman Post-Doctoral Fellowship and worked in the Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, USA. He has qualified the Tamil Nadu State Eligibility Test (SET) for Lectureship in Life Science. He has deposited around 103 nucleotide sequences in GenBank, 5 bioactive compounds in Pubchem, published 101 research and review articles including one paper in Nature Group Journal *Scientific Report* and 19 Book chapters. He has h-index of 20 with total citations of 1364 as per Google Scholar. He has edited six books on *Antimicrobial compounds: Synthetic and Natural compounds, Microbial control of Vector borne Diseases*, CRC Press, Taylor & Francis Group, New York, *Fungicides for Plant and Animal Diseases, Actinobacteria: Basics and Biotechnological applications, Algae-Organisms for Imminent Biotechnology, Microbial Biofilms – Importance and Applications* under In-tech open access publisher, Eastern Europe. He has guided 10 Ph.D. candidates and organized several national level symposia, conference and workshop programs. He received major research projects from the Department of Biotechnology, the University Grants Commission, the Indian Council for Medical research, and the International Foundation for Science, Sweden. He is member in American Society for Microbiology, North American Mycology Association, Mycological Society of India, National Academy of Biological Sciences and member in editorial boards in National, International Journals, Doctoral committee member, Board of study member in Microbiology and Reviewer in the scientific Journals and research grants. As per the reports of Indian Journal of Experimental Biology, 51, 2013, Dr. Dharmadurai Dhanasekaran is rated in the second position among the top five institutions in the field of Actinobacteria research in India.

Contributors

Sankaranarayanan, A
C.G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Surat, India

Amaresan, N
C.G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Surat, India

Adekemi Titilayo Adesulu-Dahunsi
Department of Microbiology
University of Ibadan
Ibadan, Nigeria

Kolawole Banwo
Department of Microbiology
University of Ibadan
Ibadan, Nigeria

Olatunde Samuel Dahunsi
Department of Microbiology
Landmark University
Omu-Aran, Nigeria

Cíntia Lacerda Ramos
Department of Basic Science
Federal University of Vales do Jequitinhonha e Mucuri, CEP
Minas Gerais, Brazil

Rosane Freitas Schwan
Biology Department
Federal University of Lavras CEP,
Minas Gerais, Brazil

Gözde Konuray
Cukurova University
Faculty of Agriculture, Department of Food Engineering
Adana, Turkey

Zerrin Erginkaya
Cukurova University
Faculty of Agriculture, Department of Food Engineering
Adana, Turkey

Mathews Lurth Raj, D
Department of Biotechnology
Kamaraj College of Engineering and Technology
Madurai, India

Ganesh Moorthy. I
Department of Biotechnology
Kamaraj College of Engineering and Technology
Madurai, India

Shyam Kumar, R
Department of Biotechnology
Kamaraj College of Engineering and Technology
Madurai, India

Shakena Thajuddin
Department of Microbiology
School of Life Sciences, Bharathidasan University
Tiruchirappalli, India

Selvanathan Latha
Department of Petrochemical Technology
University College of Engineering, Bharathidasan Institute of Technology (BIT) Campus, Anna University
Tiruchirappalli, India

Muthukumarasamy Arulmozhi
Department of Petrochemical Technology
University College of Engineering, Bharathidasan Institute of Technology (BIT) Campus, Anna University
Tiruchirappalli, India

Anandhan Hemamalini
Centre for Excellence in Life Sciences
Bharathidasan University
Tiruchirappalli, India

Sundarraj Dinesh Kumar
Marine Planktonology & Aquaculture Lab, Department of Marine Science, School of Marine Sciences
Bharathidasan University
Tiruchirappalli, India

Dharumadurai Dhanasekaran
Department of Microbiology, School of Life Sciences
Bharathidasan University
Tiruchirappalli, India

Antonia Terpou
Department of Chemistry
University of Patras
Patras, Greece

Anely Nedelcheva
Faculty of Chemistry and Pharmacy
Sofia University
St. Kliment Ohridski, Sofia, Bulgaria

Yunus Dogan
Buca Faculty of Education
Dokuz Eylül University
Izmir, Turkey

Khongsak Srikaeo
Faculty of Food and Agricultural Technology
Pibulsongkram Rajabhat University
Muang Phitsanulok, Thailand

Lilis Nuraida
SEAFAST Centre and Department of Food Science and Technology
SEAFAST Building Jl. Ulin No. 1
IPB University (Bogor Agricultural University)
Kampus IPB Darmaga
Bogor, Indonesia

Nilanjana Das
Bioremediation Laboratory
School of Biosciences and Technology, VIT
Vellore, India

Adikesavan Selvi
Environmental Molecular Microbiology Research Laboratory
Department of Biotechnology, Thiruvalluvar University
Vellore, India

Srinivasan Ramalingam
Department of Food Science and Technology
Yeungnam University
Gyeongsan, Korea

Ashutosh Bahuguna
Department of Food Science and Technology
Yeungnam University
Gyeongsan, Korea

Myunghee Kim
Department of Food Science and Technology
Yeungnam University
Gyeongsan, Korea

Sujatha Kandasamy
Department of Food Science and Technology
Pondicherry University
Pondicherry, India

Maria João Fraqueza
CIISA, Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine
University of Lisbon, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda
Lisbon, Portugal

Luis Patarata
CECAV, Animal and Veterinary Research Center
Universidade de Trás-os-Montes e Alto Douro
Vila Real, Portugal

Amanda L. A. Jamas
Universidade Federal Fluminense – UFF
Rio de Janeiro
Brazil

Bruno V. V. Pinto
Centro Universitário de Valença – UniFaa
Rio de Janeiro, Brazil

Amanda F. C. Estanech
Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rio de Janeiro, Brazil

Elizete Amorim
Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rio de Janeiro, Brazil

Pedro Paulo O. Silva
Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rio de Janeiro, Brazil

Romulo C. Valadão
Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rio de Janeiro, Brazil

Gesilene M. de Oliveira
Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rio de Janeiro, Brazil

Didem Deliorman Orhan
Department of Pharmacognosy, Faculty of Pharmacy
Gazi University
Ankara, Turkey

Sultan Pekacar
Department of Pharmacognosy, Faculty of Pharmacy
Gazi University
Ankara, Turkey

Mohamed Yousuff Mohamed Imran
P.G. and Research Department of Microbiology and Biotechnology
National College (Autonomous)
Tiruchirappalli, India

Nazar Reehana
P.G. and Research Department of Microbiology
Jamal Mohameed College (Autonomous)
Tiruchirappalli, India

Gangatharan Muralitharan
Department of Microbiology, School of Life Sciences
Bharathidasan University
Tiruchirappalli, India

Nooruddin Thajuddin
Department of Microbiology, School of Life Sciences
Bharathidasan University
Tiruchirappalli, India

Hasya Nazlı Ekin
Department of Pharmacognosy, Faculty of Pharmacy
Gazi University
Ankara, Turkey

Rafael Resende Maldonado
Food Department, Technical College of Campinas (COTUCA)
University of Campinas
R. Jorge FigueiredoCorrêa, Campinas, São Paulo, Brazil

Elizama Aguiar-Oliveira
Exact Sciences and Technology
Department, Santa Cruz State University
R. Jorge Amado, Ilhéus, Brazil

Eliana Setsuko Kamimura
Food Engineering Department, Faculty of Animal Science and Food Engineering
University of São Paulo, campus Pirassununga, Av. Duque de Caxias Norte
Campus da USP
Pirassununga, São Paulo, Brazil

Mônica Roberta Mazalli
Food Engineering Department, Faculty of Animal Science and Food Engineering
University of São Paulo, campus Pirassununga, Av. Duque de Caxias Norte, Campus da USP
São Paulo, Brazil

Maurizio Ciani
Dipartimento Scienze della Vita e dell'Ambiente

Università Politecnica delle Marche
Via Brecce Bianche,
Ancona, Italy

Laura Canonico
Dipartimento Scienze della Vita
e dell'Ambiente
Università Politecnica delle Marche
Via Brecce Bianche, Ancona, Italy

Francesca Comitini
Dipartimento Scienze della Vita
e dell'Ambiente
Università Politecnica delle Marche
Via Brecce Bianche, Ancona, Italy

Ilkin Yucel Sengun
Ege University, Engineering Faculty
Food Engineering Department
Izmir, Turkey

Gulden Kilic
Ege University, Engineering Faculty
Food Engineering Department
Izmir, Turkey

Nirmaladevi, R
Department of Biochemistry, Biotechnology
and Bioinformatics
Avinashilingam Institute for Home Science
and Higher Education for Women
Coimbatore, India

Section 1

Overview

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1

Diversity of Global Fermented Food Products: An Overview

A. Sankaranarayanan and N. Amaresan
C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gopal vidhya nagar, Surat, India.

1 Introduction

Fermentation is a process that transforms any substrate into food products with the aid of microorganisms such as bacteria, fungi, and yeast; enhances the quality of food materials, that is, flavor, taste, and palatability (Azokpota 2015); extends shelf life, preservation, aroma, and nutrient profile (Giraffa 2004; Katz 2012); inhibits the growth of unwanted microbes; and removes antinutritional properties. The word fermentation is derived from the Latin word *fermentare*, which means 'to leaven'. During the fermentation process, production of various organic acids, carbon dioxide, antimicrobial peptides, and bacteriocins suppresses the growth of spoilage microorganisms and the process acts as a natural preservative. The traditional practices are followed at a small-scale level in houses (curd, a fermented diary product) and at a large-scale level in industries (soy sauce, a grain-based product) in defined environmental conditions.

Fermented food is an integral part of the human diet throughout the world. The consumption of fermented food products either as a part of the diet or as a complete diet depends on the food habits of individuals. Production of fermented food may vary based on the cultures used, raw material availability, taste preferences of consumers/particular regions, and the technology used. According to a report, more than 5000 fermented food products are consumed by humans throughout the world (Ray *et al.* 2016). Although the production process may vary, all fermented food products are prepared such that they are acceptable by consumers. Moreover, in some fermented products, especially alcoholic beverages, the extended storage enhances their value than the freshly prepared one. Still some folklore practices of fermented food products are unable to reach the vicinity of scientific communities, and they are followed only by a particular hub of people at a particular time. Further, a recent research report insisted on the need of re-discovering and updating the positive aspects of fermented food products, especially the less concentrated plant-based fermented food products (Lavefve *et al.* 2019).

A variety of substrates are used in the production of fermented food products, including cereals, grains, leaves, milk, vegetables, fish, and meat, as substrates by solid-state fermentation (SSF) and submerged fermentation (SmF) processes mediated through bacteria, fungi, and yeast.

Fermented food products ensure that food is available to everyone. The reason behind this is most of the food materials available in nature are easily perishable in their raw condition, and there is a high possibility of their deterioration by spoilage microbes. Hence, enhanced quality, extended shelf life, and edibility of fermented food ensure that food is available throughout the year (Rolle and Satin 2002; Patra *et al.* 2016; Adewumi 2018) (Figure 1.1).

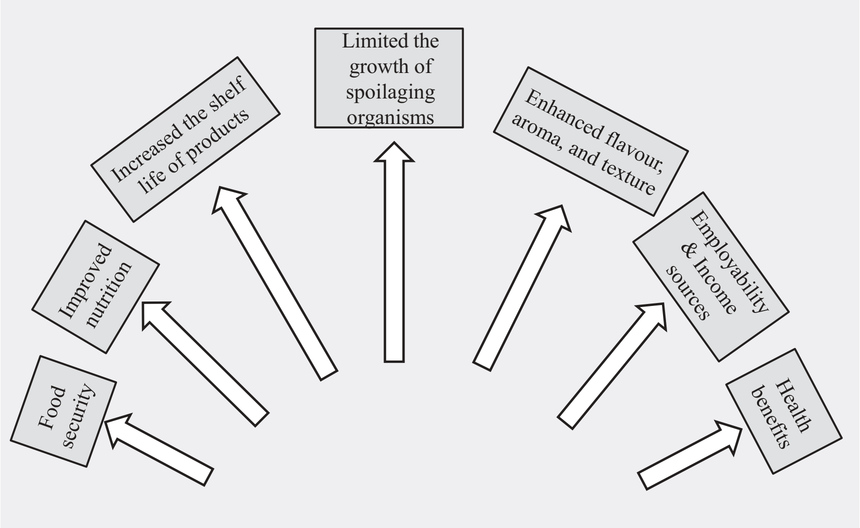
Limited the growth of spoilaging organisms
Increased the shelf life of products
Enhanced flavour, aroma, and texture
Improved nutrition
Employability & Income sources
Food security
Health benefits

FIGURE 1.1 Different beneficial perceptions of fermented food products.

TABLE 1.1

Diversified substrates used for fermented food products

S. no.	Name of the substrate	Country used	Names of the fermented food
1.	Cereal grains	Africa	Doklu
		Ghana	Kenkey
		Tanzania	Togwa
		India, Sri Lanka, Nepal	Dosa and idli
2.	Legume	Japan	Natto
		India	Kinema
		Indonesia	Tempe
3.	Roots and tubers	West Africa	Lafun
		Brazil	Taruba
4.	Fruits and vegetables	Korea	Kimchi
		Nepal	Khalpi
		Mexico	Pulque
		Taiwan	Suan-tsai
5.	Bamboo shoots	Taiwan	Jiang-sun
		India (Northeast)	Soidon
6.	Milk and dairy products	China	Kurut
		Russia	Koumiss
		Turkey	Aryan
		India	Shrikhand
7.	Meat and sea foods	Portugal	Alheira
		Turkey	Sucuk
		Thailand	Nham
		Ethiopia	Wakalim
		India	Jamma

(*Continued*)

TABLE 1.1 (Cont.)

S. no.	Name of the substrate	Country used	Names of the fermented food
8.	Tea leaves	Thailand China	Miang Pu-erh Kombucha
9.	Cocoa	Venezuela Indonesia West Africa	Chocolate
10.	Sugar cane	Philippines Brazil	Basi Cachaca
11.	Palm	Camaroon	Palm wine
12.	Banana	Tanzania Burundi	Mbege Isongo
13.	Honey	Ethiopia	Tej
14.	Carrot	India	Kanji

Another noteworthy point is that the availability of diversified substrates, including cereal grains, pulses, roots and tubers, fruits and vegetables, edible bamboo shoots, milk and dairy products, meat and sea foods, tea leaves, cocoa, sugar cane, and oil palm sap, is the reason for the availability of a variety of fermented products in the world (Table 1.1).

The purpose of this chapter is to introduce the various fermented food products and their diversity, including household fermented food products, countries where they are used and their health benefits. It is interesting to note at this juncture that although several books, reviews (Hesseltine and Wang 1986; Reddy *et al.* 1986; Nout and Rombouts 1990; Sanni 1993) and reports/monographs are available on indigenous food products, still the role of microbes in the production of few of the indigenous fermented food products is not yet revealed (Beuchat 1983, 1987; Reddy *et al.* 1986). Hence, researchers/scientists involved in the research based on fermented food products should reveal the role of organisms involved in the formation of fermented food products and list out various health aspects that one may gain by consuming them. There are chances that the fermented food products have some indigenous features that are beneficial for human health, and it is a bounden duty of researchers/scientists to report about this to the world.

2 Food Fermentation—Definition and Types

Fermentation is a basic and traditional practice followed everywhere in the world from a household scale to industrial level. Fermented foods are gifts to humankind by nature, and the reactions are mediated by microbes.

Fermented food is defined as the metabolic conversion of raw substrate into food with improved quality, and the reaction is mediated by microorganisms. In another view, it is a metabolic process in which carbohydrates/associated compounds are oxidized with the release of energy in the absence of electron acceptors.

Fermented food is also defined as a process that utilizes the microbial metabolic activity for the stabilization and transformation of food materials (Terefe 2016) fit for consumption with enhanced values of the product.

It is a metabolic-oriented microbial process that enhances the shelf life of the product, its aroma, flavor, and texture, and inhibits the unwanted microbes and removes toxicity from the food and makes it ready for consumption. Katz (2012) stated that 'the fermented food products are the transformation of food by microbial mediated enzymes.'

SSF and SmF processes are carried out in fermented food industries in the presence as well as in the absence of oxygen. SSF process includes mushroom, bread, cocoa, tempeh (Erkmen and Bozoglu 2016), taruba (Ramos *et al.* 2015), and gari (Chisti 2014), whereas submerged fermentation (SmF) process includes pickling vegetables, yogurt, beer, wine (Erkmen and Bozoglu 2016), busa (Blandino *et al.* 2003) fufu, lafun, and agbelima (Oyewole 2001; Coulin *et al.* 2006).

3 Global Diversity of Various Fermented Food Products

Fermentation is one of the traditional and ancient processes, in which the metabolic activities of microbial organisms (Terefe 2016; Wilburn and Ryan 2017) enhance the value of the food products (Nout 2014). By the end of the fermentation, antimicrobial metabolites, production of bacteriocins, and synthesis of organic acids inhibit the food-borne pathogens (Kim *et al.* 2016) and enhance the organoleptic characteristics of food, thereby increasing its health benefits and shelf life (Heperkan 2013; Hwang *et al.* 2017). Basically, fermented food is an integral part of everyday diets of humans around the world and plays an important role in various traditional practices (Ansorena and Astiasarán 2016; Narzary *et al.* 2016; Kanwar and Keshani 2016) (Table 1.2).

Basically, a wide variety of fermented foods is available and used by all kinds of people invariably, especially those living in marginalized and vulnerable societal platforms (Montet *et al.* 2006). Some of the fermented food products play an important role in the traditional rituals of a particular community. Fermented milk products play an important role in the human diet throughout their life (Adolfsson *et al.* 2004). Lactic acid bacteria (LAB) play a predominant role in the production of fermented food products. LAB generate acidic environment, which inhibits the spoilage and disease-causing organisms by producing bacteriocins substances (Widyastuti and Febrisiantosa 2014). Also, the presence of vitamins, calcium, potassium, magnesium, and zinc in milk and milk-based fermented products is a noteworthy point. The presence of vitamins varies according to the fermented dairy products (Dönmez *et al.* 2014).

Fermented cereal-based foods basically lack essential amino acids and other basic nutritive compounds. A simple and alternate way mediated through fermentation is to increase the nutritive value of cereals (Ozdemir *et al.* 2007), especially some amino acids, vitamins of group B (Blandino *et al.* 2003), antioxidants (ĐorŤevic *et al.* 2010), and free amino acids, and to reduce the nonnutritive compounds, such as polyphenols, phytates, and tannins (Şanlier *et al.* 2017).

Fermented milk and milk-based products are part of the human diet throughout their life. Thus, needless to reveal the importance of dairy and dairy-based fermented food products (Adolfsson *et al.* 2004) on human health. LAB play a vital role in the production of various milk and milk-based fermented products (Żukiewicz-Sobczak *et al.* 2014), in enhancement of their nutritional properties (Jeong *et al.* 2016), and also in improving their shelf life (Widyastuti and Febrisiantosa 2014). LAB enhance the value of fermented milk and milk-based products, providing an acidic environment by the conversion of lactose into lactic acid, which inhibits both the spoilage and pathogenic microbes (Widyastuti and Febrisiantosa 2014). Cheese is a prominent fermented dairy product rich in calcium and vitamin B contents (Ansorena and Astiasarán 2016). According to a recent report, the breakdown of casein and the formation of bioactive compounds are responsible for the biological activities (López-Expósito *et al.* 2017). A probiotic coagulated fermented dairy product yoghurt has enhanced vitamin B series with calcium, magnesium, potassium, and zinc ions (Wang *et al.* 2013). Microbes, especially LAB, play a vital role in the aroma and taste of fermented dairy products, especially koumiss. Koumiss is a slightly alcoholic fermented beverage with sour taste and alcoholic flavor (Lv and Wang 2009; Zhang and Zhang 2012; Choi 2016), which is due to the lactic acid and alcohol fermentation. The last product in

TABLE 1.2

List of various fermented food products of the world

Sources	Substrates	Product	Nature	Organisms involved	Country	Health benefits/nutrition	Reference
Cereal	Rice and black gram	Idli, Dosa	Slight acidic, soft, moist Thin, shallow fried/soft	*L. delbrueckii*, *L. fermenti*, *B. amyloliquefaciens*, *S. cerevisiae*, *C. pseudotropicalis* *C. tropicalis* *E. faecalis*	India, Sri Lanka, Malaysia, Singapore, Nepal India, Sri Lanka, Malaysia, Singapore, Nepal	Carbohydrates, dietary fiber, protein Less saturated fat; rich with iron and calcium	Sridevi *et al.* 2010 Soni *et al.* 1986
	Maize, Sorghum	Busa	Beverage	*S. cerevisiae*, *L. plantarum*, *L. casei*, *L. Brevis*	Kenya, East Africa	Sour taste; sugar and protein	Odunfa and Oyewole 1997
	Wheat	Injera	Bread-like product, slightly acidic	*L. plantarum*, *S. cerevisiae*	Ethiopia	Iron, potassium, vitamin A and K	Olasupo *et al.* 2010
	Sorghum	Hussuwa	Cooked dough	*L. fermentarum*,	Sudan	Antioxidant, antidiabetic, and antiobesity	Yousif *et al.* 2010
	Wheat flour	Jalebi	Sweet, crispy, soft in nature, chewy; ghee fried	*L. fermentarum*, *L. lactis*, *S. cerevisiae*	India, Pakistan, Nepal	Lactose and protein with fat	Batra and Millner 1976; Asgar and Chauhan 2019
	Sorghum, wheat, and millet	Kunu-zaki	Slightly acidic, viscous	*Corynebacterium* sp. *Fusarium* sp. *S. cerevisiae*	Nigeria	Nonalcoholic	Oguntoyinbo *et al.* 2011
	Rice	Lao-chao	Paste, glutinous	*R. oryzae*, *Sacchromycopsis* sp.	China	Rich with protein, fiber, and unsaturated fat	Blandino *et al.* 2003
	Maize	Poto-poto	Slurry	*L. plantarum*, *L. acidophilus*, *Bacillus* sp. *Enterococcus* sp.	Congo	Multivitamins and minerals	Abriouel *et al.* 2006
	Maize	Pozol	Slightly acidic,	*Strep. bovis*, *Strep. Macedonicus*	Mexico	Starch with protein, lipid, and less fat	Díaz-Ruiz *et al.* 2003

(*Continued*)

TABLE 1.2 (Cont.)

Sources	Substrates	Product	Nature	Organisms involved	Country	Health benefits/nutrition	Reference
			highly viscous				
	Rice	Puto	Steam based	*Leuconstoc mesenteroides*	Philippines	Carbohydrate rich with sodium and potassium	Steinkraus 2004
	Rye, wheat	Sourdough	Slightly acidic	*Lb. casei*, *Lb. delbrueckii*, *Lb. Plantarum*	Australia, America, Europe	High protein, essential amino acids, and fiber	Pontonio and Rizzello 2019
	Maize, Sorghum, Millet	Uji	Sour, acidic	*L. plantarum*, *Leuconstoc mesenteroides*	Uganda, Kenya	Starch and dietary fiber	Odunfa and Oyewole 1997
	Maize	Champu	Beverage	*S. cerevisiae*	Colombia	Vitamins, minerals, dietary fiber, antioxidant	Osorio-Cadavid *et al.* 2008
Milk	Cow milk/ buffalo milk	Cheese	Solid, soft	*Lc. lactis*, *Lb. casei*, *Leuconostoc* spp. *Peni. Roqueforti*	Throughout Universe	Protein, total fat, cholesterol, sodium, potassium, and calcium	Quigley *et al.* 2011
	Cow milk	Amasi	Sour taste, acidic, thick fluid	*Enterococcus* sp.*Leuconostoc* sp.	Zimbabwe, South Africa	Antiinflammatory, boost the immune system, and reduce inflammation	Osvik *et al.* 2013
	Cow/buffalo milk	Dahi	Gelly with whey	*Lact. lactis*, *Strep. cremoris*, *Candida* sp.	India, Nepal, Sri Lanka, Bangladesh, Pakistan	Boost the immune system, lower cholesterol, promote digestion	Patil *et al.* 2010
	Sheep, goat, cow	Kefir	Fermented milk, alcoholic	*Lb. brevis*, *Lb. bulgaricus*, *S. cerevisiae*	Russia	Improved digestion, hypocholesterolemic effect, antiinflammatory, antioxidant	Bernardeau *et al.* 2006; Rosa *et al.* 2017
	Animal milk	Yogurt	Acidic, viscous, curd like gel	*Strep. thermophilus*, *Lb. acidophilus*, *Bifidobacterium* sp.	America, Australia, Europe	Maintain bone health, rich with calcium, vitamins, and minerals; conjugated linoleic acids	Angelakis *et al.* 2011
	Cow milk	Viili	Dense liquid, sweet	*Lb. lactis*, *Leuc. Mesenteroides*	Finland	Antioxidant, anti inflammation, immuno modulation, anticancer	Kahala *et al.* 2008
	Cow milk	Doogh/ Ayran/Tan	Diluted yoghurt	*Lb. acidophilus*	Turkey, Iran, Jordan, Armenia, Kazakhstan	Rich with calcium, minerals, and vitamins	Yildiz, 2010

	Cow milk/ buffalo milk	Borhani	Diluted Yoghurt	*Lb. acidophilus*, *Lb. lactis*	Bangladesh	Rich with calcium, minerals, and vitamins	Nahidul-Islam *et al.* 2018
	Goat milk	Rubing milk cake	Acidic	*Lactobacillus* sp. *Lactococcus* sp.	China	Contains lactose and galactose	Liu *et al.* 2018
	Cow milk	Rob	Beverage	*Lb. fermentum*, *Lb. acidophilus*, *Lactococcus lactis*, *Streptococcus salivarius*; *S. cerevisiae*, *Candida kefyr*	Sudan	Antioxidant, reduce blood pressure, and lower the cholesterol	Abdelgadir *et al.* 2001
Vegetables	Red onion	Hom-dong	Fermented onion	*Leuc. mesenteroides*, *Lb. plantarum*, *Lb. fermentum*	Thailand	Organosulfurs, phenolics, flavanoids, saponins, and sapogenins	Phithakpol *et al.* 1995
	Cabbage, pepper, ginger	Kimchi	Acidic, slightly sour	*Leuc. mesenteroides*, *Lb. plantarum*, *Pichia*	Korea	Antiinflammatory, immune system promotion, and cholesterol reduction	Jung *et al.* 2011; Hongu *et al.* 2017
	Cucumber	Oiji Jiang-gua	Salty Sweet/salty	*Leuc. mesenteroides*, *Lb. brevis*, *Lb. Plantarum*, *Pediococcus pentosaceus* *Leuc. mesenteroides*, *Leuc. lactis*, *Lb. pentosus*	Korea Taiwan Taiwan	Dietary fiber, antioxidant, potassium, and vitamins Antibacterial potential	Alexandraki *et al.* 2013; Chen *et al.* 2012; An-wei *et al.* 2010; Chen *et al.* 2012
	Turnip	Sunki	Acidic, sour	*Lb. sunkii*, *Lb. rapi*, *Lb. plantarum*	Japan	Carbohydrate, protein, and dietary fiber	Watanabe *et al.* 2009; Endo *et al.* 2008
	Mustard leaves	Burong mustasa Dakguadong	Salty Salty	*L. brevis*, *Pediococcus cerevisiae* *L. plantarum*	Philippines Thailand	Dietary fiber, vitamins, and minerals Fiber, protein, sodium, and vitamins	Larcia *et al.* 2011; Di Cagno *et al.* 2013; Panghal *et al.* 2018
	Mustard/beet	Dua-muoi /Dua chua/ Dua cu cai (beet)	Salt and sugar	*L. plantarum*, *L. pentosus*, *L. fermentum*	Vietnam	Dietary fiber, vitamins, potassium, and calcium	Nguyen *et al.* 2013; La Anh 2015
	Egg plant	Ca muoi	Salt and sugar	Lactic acid bacteria	Vietnam	Dietary fiber, vitamin, folate, and copper	La Anh 2015
	Coconut press cake	Tempe bongkrek*	Soft and solid form; slightly salty	*Rhizopus* sp.	Indonesia	Protein, fiber, water-soluble vitamin	Shah and Singhal 2017

(*Continued*)

TABLE 1.2 (Cont.)

Sources	Substrates	Product	Nature	Organisms involved	Country	Health benefits/nutrition	Reference
	Carrot and beetroot	Kanji	Salty drink	Lactic acid bacteria	India	Carbohydrate, protein, sugars; Indigestion and liver disorder	Lamba *et al.* 2019
	Mixed vegetables	Yan-cai	Salty	*Lactobacillus*, *Leucobacter*	China	Contains lactic acid and acetic acid	Liu *et al.* 2018
	Pak-sian	Pak-sian-dong	Salt and sugar	*Lb. brevis*, *Lb. plantarum*	Thailand	Carbohydrate and sugar	Tanasupawat and Komagata 1995
	Cauliflower/cucumber/cabbage/celery	Torshi/Tursu	Salty	*Lb. plantarum*	Turkey, Bulgaria, Croatia, Serbia	Carbohydrate, iron, sodium, magnesium, manganese, and potassium	Cetin 2011
	Ensete ventricosum	Kocho	Soft and solid form	*Bacillus larvae*, *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus* sp. *Clostridium* sp.	Africa	Carbohydrate, protein, and fiber	Gashe 1987; Yirmaga 2013
	Tea leaves	Kombucha	Slightly sweet, acidic, and refreshing beverage	*Komagataeibacter xylinum, Acetobacter xylinoides, Gluconobacter oxydans, Gluconacetobacter hansenii, Oenococcus oeni, Komagataeibacter europaeus, Lactobacillus sp. Saccharomyces sp., Schizosaccharomyces pombe, Zygosaccharomyces kombuchaensis Torulaspora delbrueckii, Brettanomyces sp*	China, Japan, Russia, Europe, France, Germany, North Africa, Switzerland	Neurodegenerative disease prevention, blood pressure reduction, antioxidant activity, hypoglycemic effect, detoxification activity, and anticancer properties	Villarreal-Soto *et al.* 2019; Sinir *et al.* 2019
Legume	Locust bean	Dawadawa	Alkaline, sticky	*B. licheniformis*, *B. subtilis*, *B. amyloliquefaciens*	Nigeria, Ghana	Protein and crude fiber	Meerak *et al.* 2008
	Bengal gram	Dhokla	Soft, steamy, and spongy	*Ent. faecalis*, *Lb. Fermenti*	India	Protein, dietary fiber, carbohydrate	Blandino *et al.* 2003
	Soybean, red pepper	Gochujang		*B. licheniformis*, *B. subtilis*, *B. amyloliquefaciens*	Korea	Carbohydrate and dietary fiber	Shin and Jeong 2015
	Soybean	Tempeh	Solid cake form	*Rhizopus*; Lactic acid bacteria and yeast	Indonesia, Japan	Protein, carbohydrates, and dietary fiber	Efirwati Suwanto *et al.* 2013
	Soybean	Shuidouchi	Salty	*Bacillus* sp. *Bacteroides* sp. and *Lactobacillus* sp.	China	Proteins, vitamins, and minerals	Chen *et al.* 2018

Root crop	Cassava	Chikwangue	Staple	*Bacillus* sp, *Lactobacillus* sp, *Corynebacterium* sp.	Africa, Zaire	Phytic acid, zinc, calcium, and iron	Odunfa and Oyewole 1997
	Cassava	Fufu	Staple	*Bacillus* sp, *Lb. brevis*, *Lc. lactis*, *Lb. Bulgaricus*	West Africa	Red blood cell production, muscle functioning	Odunfa and Oyewole 1997
	Cassava	Tape	Sweet dessert	*Streptococcus* sp. *Rhizophus* sp.	Indonesia	Rich with calcium, magnesium	Ohba *et al.* 1989
Meat	Pork/ostrich	Salami	Spicy and salty	*Staphylococcus xylosus*/*Micrococcus*/ *Lactobacillus sake*, *Lactobacillus curvatus*	Europe	Carbohydrate, saturated fat, mono saturated, and poly unsaturated fat	Aquilanti *et al.* 2016; Bohme *et al.* 1996
	Beef/mutton	Sucuk/ Soudjouk	Spicy and salty	Lactic acid bacteria/*Staphylococcus* sp. *Micrococcus* sp.	Turkey	Low cholesterol, high level of poly unsaturated fatty acid	Akkaya *et al.* 2014; Eren *et al.* 2008; Yüceer and Tuncer, 2015; Baggio *et al.* 2002
	Beef/buffalo	Pastirma/ Pastrami	Dry fermented sausage	*Lb. sake, Lb. plantarum, Lb. curvatus, and Lb. brevis*	Turkey	Antioxidant, rich amino acids	Aksu *et al.* 2016; Kaban 2013; Akköse *et al.* 2016
	Pork/beef	Alheira	Dry/semi-dry sausage	*Lb. plantarum*, *Lb. paraplantarum*, *Lb. brevis*, *Lb. sakei*	Portugal	Carbohydrates, minerals, less fat, and cholesterol	Campos *et al.* 2013
	Pork	Sa-um	Semi-dry, gummy	*Firmicutes*, *Proteobacteria, Bacteroidetes, C. butyricum*, *C. citroniae*, *C. methylpentosum*, *C. perfringens*, *C. saccharogumia C. tetani*	India (North eastern)	Carbohydrate, protein, and lipids	De Mandal *et al.* 2018
Fish	Fish	Ngari/Shidal	Slightly acidic and salt free	*Lb. plantarum, Lb. pobuzihii, Lb. coryniformis, B. subtilis Staphylococcus carnosus*	India (North eastern)	Antioxidant, DPPH radical scavenging activity, calcium, potassium, magnesium, sodium	Majumdar *et al.* 2015. Muzaddadi and Basu, 2012
	Fish	Hentak	Slightly acidic and salt free	*Micrococcus* sp. *Staphylococcus* sp., and lactic acid bacteria	India (North eastern)	Antioxidant, DPPH radical scavenging activity, calcium, potassium, magnesium, and sodium	Majumdar *et al.* 2015.
	Fish and shrimp	Prahok (fish), Kapi (shrimp) and toeuk tray (fish)	Paste form/ sauce; salty	*Bacillus* sp*, Clostridium* sp*, Staphylococcus* sp, *and Tetragenococcus* sp.	Cambodia	Amino acid, crude fat, total nitrogen	Chuon *et al.* 2013

(*Continued*)

TABLE 1.2 (Cont.)

Sources	Substrates	Product	Nature	Organisms involved	Country	Health benefits/nutrition	Reference
	Fish/shrimp/ oyster	Bagoong	Paste/salty	*Bacillus, Micrococcus,* and *Staphylococcus*	Philippines	Good appetizer and seasoning agent	Smague 1994
	Fish	Dang-pui-thu		*B. subtilis, Staphylococcus simulans, Streptomyces sp.*	India (North eastern)	Protein rich, amino acids, fat, and minerals	Singh *et al.* 2014
	Fish	Nuocmam, patis, mampla	Liquid form; Sauce	Facultative anaerobic bacteria	Philippines	Rich with proteins and amino acids, fat and total nitrogen	Smague 1994
Fruits	Breadfruit	Poipoi	Beverage/ dry form	Lactic acid bacteria	Tahiti	Rich with starch and a good energy source	Aalbersberg *et al.* 1988; Jones *et al.* 2011
	Red grape	Hardaliye	Beverage	*Lb. plantarum*	Turkey	Antioxidant property	Kilic *et al*. 2016; Coskun and Tirpanci Sivri, 2013
	Grape	Wine	Alcoholic beverage	*S. cerevisiae*	Throughout the World	Antioxidant, boost immunity, and increase bone density	German and Walzem 2000
	Olive	Olive oil	Oil	*Lb. plantarum*, *Lb. brevis*, *Lb. pentosus*, *Lb. mesenteroides*	Spain, Italy	Carbohydrate, sugar, protein, and dietary fiber	Sherahi *et al*. 2018
	Durian	Tempoyak	Condiment/ ingredient for cooking	*Lb. plantarum*	Malaysia	Carbohydrate, protein and dietary fiber	Amiza *et al.* 2006
	Cummingcordia	Pobuzihi	Cake/granular form	*Lb. pobuzihii, Enterococcus casseliflavus*, and *Weissella cibaria*	Taiwan	Rich with carbohydrates	Chen *et al.* 2013b; Swain *et al.* 2014
	Peaches	Yan-taozih	Salty and sweet	*Leuconostoc mesenteroides*,	China and Taiwan	Protein, dietary fiber, and vitamin C	Chen *et al.* 2013a
Miscellaneous items	Coconut water	Nata de coco	Dessert	*Acetobacter xylinum*	Philippines and Japan	Thickening and gelling agent; used for food packaging	Verschuren *et al.* 2000
	Honey	Tej/Kuri	Sweet, effervescent dense liquid	*S. cerevisiae, Kluyveromyces bulgaricus*	Ethiopia, Eriteria/Cameroon	Organic acids, esters, alcohol, volatile fatty acids, carbonyl compounds, and volatile phenols	Yohannes *et al.* 2013

* Consumed within 1–2 days; not consumed if it is yellow in color. Indonesian Government outlawed the production; however still in use by poor

Lb.—Lactobacillus

our list, kefir, is rich in vitamins and minerals. It is an acid-fermented drink, which is mildly alcoholic, creamy, and sour in taste (Prado *et al.* 2015; Rai *et al.* 2017).

A variety of products are available as fermented vegetables, with a special mention of kimchi (Korea) and sauerkraut (China), consumed throughout the world. Kimchi was one among the five healthiest foods in the world ranked by food magazine (2008), and thus grabbed worldwide attention (Hong *et al.*, 2016a, 2016b; Kim *et al.* 2017). Sauerkraut, a cabbage-based fermented vegetable product, is being used in China for many years. Basically, vitamins, minerals, phosphorous, and phenolic compounds present in sauerkraut yield a lot of health benefits, and the health benefit level enhances after fermentation (Xiong *et al.* 2014).

In comparison with the fermented vegetable products, the fermented fruit products are less. However, they also have rich nutritional and health benefits. The pulp of durian fruit is used as a substrate in making salted fermented fruit product, namely tempoyak in Malaysia (Leisner *et al.* 2001). Sweet cherry (*Prunus avium* L.) is one among the widely used fermented food products in Italy, the United States, Iran, and Turkey.

Fermented legumes (Todorov and Holzapfel 2014) and root crops are also consumed throughout the world. After fermentation, beneficial features such as nutrient values are enhanced, while unfavorable features are diminished in legume-based fermented food products (Frias *et al.* 2017). Legume-based fermented food products are used in the form of soup, paste, and sticky material. In the fermentation process of legume-based products, especially soybean, both bacteria and filamentous fungi are involved (Meerak *et al.* 2007; Dajanta *et al.* 2009; Tamang 2010). Regarding root crops, cassava is the traditional and widely used staple food.

Fermented meat-based products are also one among the important food products (Kumar *et al.* 2017). The features of the meat products may enhance during fermentation because of the physicochemical, biochemical, and microbiological changes by key organisms involved in their production. Owing to the increasing awareness of safe and healthy fermented food products, inclusion of probiotic cultures has added a lot of benefits to fermented meat products (Neffe-Skocińska *et al.* 2016). LAB and micrococci used in the production of Turkish-based meat product 'sucuk' enhance its taste, soft texture, and color (Kaban and Kaya 2008).

Owing to quick perishability of fresh fish, the concept of fermentation is very popular in fermented fish products. Besides this benefit, the enriched nutritional value, aroma, and taste add flying colors to the fermented fish products (Özyurt *et al.* 2016; Adjou *et al.* 2017).

Fermented beverages are an integral part of the human diet and attract the attention of consumers because of the presence of various nutritive values present in them (Baschali *et al.* 2017). This explains the wide consumption of alcoholic beverages at this juncture, especially wine and beer. A special mention over here is Boza juice widely used in Turkey, which is prepared from a variety of cereals and is used during winter and autumn seasons. Shalgam juice, also from Turkey, is prepared from Bulgur flour, with black carrots as a base material along with salt (Üçok and Tosun, 2012). Both bacteria and fungi play an important role in its preparation (Erten *et al.* 2008). In general, in the fermented drinks, the low pH and other unfavorable features reduce the growth of spoilage and other unwanted bacterial populations. Furthermore, various vitamins, proteins, amino acids, and minerals are produced during the fermentation process (Colak *et al.* 2012).

Miscellaneous fermented foods, such as kanji prepared from rice and carrot, palm wine from palm sap, and Tej from sugar cane, also play a pivotal role in human diet and health.

4 Health Benefits

World Health Organization (WHO) and Food and Agriculture Organization (FAO) have recommended the consumption of healthy fresh foods prepared from fruits and vegetables to prevent various diseases (Endrizzi *et al.* 2009). By consuming fermented food products, various ailments are prevented, and further during the fermentation process, these food products enhance various health-associated components. The harboring of microbiota in fermented food (Marco *et al.* 2017) is the main reason behind its benefits. Of course, the beneficial microbiota and trendsetting probiotic bacteria reach the lower intestinal tract of human through the fermented food material; however, how long can these fermented food microbes overcome with the gut flora of human is still unclear and needs more attention in future research aspects (Derrien and van Hylckama Vlieg 2015), although the microbiota enhance the nutritive, commercial value and yield lot of benefits to human health.

In fermented food products, probiotic bacteria play a pivotal role. However, the exact mechanism of probiotic bacteria is yet to be revealed (Terpou *et al.* 2018). Because of biogenic effects, various primary metabolites are produced during the fermentation process. Fermented food stuffs may induce natural immunity, immunomodulatory activity, and enhance the value of final products. Probiotic bacteria can act as a prominent candidate to especially provide health benefits. Probiotic bacteria-based fermented foods act against allergy, cancer, pathogenic microbes and are rich in antioxidant properties (Caggianiello *et al.* 2016; Khalil *et al.* 2018).

Dairy and dairy-based products are an integral part of the human diet throughout the world for many years. Per capita milk consumption globally averages 214 liters per year (FAO 2006). It has been reported that (FAO 2013; Agyei *et al.* 2019) milk contains numerous nutrients from proteins to minerals, such as calcium, magnesium, and selenium. Nutritive value of fermented dairy products depends on various factors, such as age of animal, health, feed, and seasonal variation (Laben 1963; Bansal *et al.* 2003; Walker *et al.* 2004; Jenkins and McGuire 2006). LAB, because of their proteolytic activity, release the amino acid from the fermented dairy products (Pessione and Cirrincione 2016), which is highly required for human metabolism. Conjugate linoleic acids (CLAs) and sphingolipids fight against cancer, and CLA especially found in cheese helps to reduce obesity. Reports reveal that koumiss was used to treat tuberculosis, ulcers, and hepatitis in Mongolia (Wu *et al.* 2009). Owing to lot of beneficial features, including antifungal activity against *A. flavus* (Gamba *et al.* 2016), focus has increased toward kefir in recent years (Leite *et al.* 2013; Nielsen *et al.* 2014; Rosa *et al.* 2017).

In some fermented products, the microbes involved in the fermentation process produce bacteriocins and prevent the growth of spoilage and pathogenic organisms. Hence, they indirectly play a role in health benefits and help in anticancer activity, fighting with cholesterol and intestinal infections, accelerating the immune response, and providing high vitamins and minerals in olive fermentation. In this, it is reported that the abundance of oleic acid has a fruitful control effect on different types of cancer such as prostate, breast, and colon (Sales-Campos *et al.* 2013; Peres *et al.* 2017). The presence of COX-inhibitor has the capacity to fight against neurodegenerative disorders (Peres *et al.* 2017). Miso, a soybean-based fermented product, protects the consumer from radiation, stroke, hypertension, and some types of cancer (Ohuchi *et al.* 2005; Watanabe *et al.* 2006; Watanabe 2013). Cardiovascular disease and hypertension are prevented by the secretion of nattokinase enzyme, which is produced by *Bacillus subtilis natto* in the fermented soybean product ‘Natto’ (Park *et al.* 2012; Hitosugi *et al.* 2015; Şanlier *et al.* 2017). Fermented brown rice reduces DNA damage because of oxidative stress (Kong *et al.* 2015), and it is considered as a functional food product because of its various beneficial features (Kataoka *et al.* 2008; Ilowefah *et al.* 2015; Onuma *et al.* 2015). Boza, a traditional cereal-based nonalcoholic beverage, is rich in minerals; hence, it is a nourishing drink containing angiotensin-converting enzyme inhibitory peptides (Kancabaş and Karakaya, 2013). Shalgam juice is believed to control colon cancer.

In tubers and root-based food substances, such as cassava, potatoes, cocoyam, and yam, the main carbohydrate sources are hydrolyzed by LAB during the fermentation process and lead to lactic acid, which creates an acidic environment. Thus, the other pathogenic microbes and organisms responsible for food-borne illnesses were avoided similar to how naturally protected fermented food benefited the health of consumers (Giraffa 2004). *Ogi*, a cereal-based fermented food, is considered as the best infant food in West Africa (Odugbemi *et al.* 1991) because of its efficient inhibition activity over *S. typhi* and *S. paratyphi*. Likewise, 'DogiK' is used as a medicine to control infant diarrhea in Nigeria (Olukoya *et al.* 1994).

Microbes involved in the production of fermented food products not only provide health benefits but also secrete a lot of metabolites, which enhance the organoleptic characteristics of fermented food products (Akissoe *et al.* 2015; Blanco *et al.* 2016). Recent research on antibacterial (Bhattacharya *et al.* 2016, 2018; Sknepnek *et al.* 2018), antifungal, (Mahmoudi *et al.* 2016) antiviral (Fu *et al.* 2015), and antioxidant properties (Sun *et al.* 2015; Gamboa-Gomez *et al.* 2016, 2017) of plant-based fermented beverage 'Kombucha' states that the antioxidant property is mediated by microbial metabolism of tea polyphenols (Chakravorty *et al.* 2016). This report has been confirmed by performing both *in vitro* (Gamboa-Gomez *et al.* 2017; Vazquez-Cabral *et al.* 2017; Sknepnek *et al.* 2018) and *in vivo* (Bellassoued *et al.* 2015; Gamboa-Gomez *et al.* 2017; Salafzoon *et al.* 2017; Pakravan *et al.* 2017) tests.

A recent report revealed that the beneficial activity (Xie *et al.* 2018; Berretta *et al.* 2018) of coffee beans was due to the presence of polyphenols and melanoidins (Godos *et al.* 2014; Lopez-Barrera *et al.* 2016).

5 Conclusion

Microbes are involved in the production of fermented food products, which render numerous health benefits to humans. The results of the report are encouraging and anchor the beneficial activities of fermented food. Interestingly, recent research is based on *in vivo* studies using animals and cell lines. In the near future, the course of research may be anchored and focused toward *in vivo* studies, especially the competition between fermented microbes and human gut micro biota and concentration toward traditionally fermented food products produced by various innovative scientifically unrevealed methods. These approaches may lead to several beneficial scientific outcomes for humankind.

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Section 2

Traditional Fermented Food Products

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2

Traditional Fermented Foods of Nigeria

Adekemi Titilayo Adesulu-Dahunsi
Department of Microbiology, Landmark University, Omu-Aran, Nigeria

Samuel Olatunde Dahunsi
Department of Microbiology, Landmark University, Omu-Aran, Nigeria

Kolawole Banwo
Department of Microbiology, University of Ibadan, Ibadan, Nigeria

1 Introduction

Fermented cereals and cassava tubers produced by lactic acid fermentation, fermented alcoholic beverages from alcoholic fermentation, and many other fermented African vegetable condiments from traditional alkaline fermentation constitute essential diets of many Nigerians. These fermented foods are culturally and socially acceptable by many. They contribute to sustainable development in Nigeria and many developing countries of Africa, thus improving the livelihoods of the citizens, increasing the nutritional value of the foods, proposing empowerment initiatives among the rural dwellers, alleviating poverty, and enhancing food security and income generation (Adesulu and Awojobi, 2014).

Fermentation techniques have been indigenously developed for a wide range of traditional fermented foods of Nigeria. The spontaneous fermentation of these foods is an age-long food culture, which is largely practiced in many Nigerian homes, as the tropical climate provides suitable temperatures for their production. Several fermented foods are produced on a small- or medium-scale level in Nigeria, including *gari, fufu, lafun, abacha, ogi, iru, ogiri, ugba, owoh, okpehe, nunu, wara, emu, pito, dolo, fura, burukutu, sekete, kunun-zaki, agadagidi, masa*, and *kokoro*. These fermented foods are the hub of consortia of microorganisms, as they are naturally present as microbiota in the raw substrates or immediately after the fermentation process is initiated (Olasupo et al., 2010; Adesulu-Dahunsi et al., 2018a). Many traditional foods produced by fermentation serve as the main course meal, snacks, condiments, and beverages (Sanni and Adesulu, 2013).

In Nigeria and other West African countries, fermented foods from plant sources constitute a significant portion of the protein intake and are also a cheap source of energy (Uzogara et al., 1990). Common fermented foods and beverages in Nigeria are derived from plants that are grown in various localities, and several beneficial microorganisms have been reported to be associated with these foods (Odunfa and Oyewole, 1997; Olasupo et al., 2010; Adesulu-Dahunsi et al., 2017a, 2018b). Cereals such as maize, sorghum, millet, and cassava tubers cultivated in Nigeria are traditionally fermented into staple foods as well as weaning foods for infants, and the fermentation process is mostly dominated by lactic acid bacteria (LAB) species (*Lactobacillus*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, and *Weissella*) and few yeast species (*S. cerevisiae*, *Kluyeromyces*, *Debaryomyces*, and *Aspergillus*) (Agarry et al., 2010; Chelule et al., 2010; Adesulu-Dahunsi et al., 2017b). LAB species primarily constitute native population in many Nigerian indigenous fermented foods and cereal beverages, and they play key roles in safety and acceptability of the final products, as the acid produced gives the desired flavor and sourness to the food. Autochthonous *B. subtilis* have been reported to perform essential roles during spontaneous fermentation of condiment; strains of *Bacillus cereus* have been reported to be involved during fermentation of *okpehe* (Oguntoyinbo et al., 2007). Some *B. subtilis* strain from fermented condiments can also produce λ-polyglutamic acid, which gives characteristic texture to the food end products (Urushibata et al., 2002; Nishito et al., 2010).

In this chapter, an attempt has been made to explicitly review traditional fermented foods and beverages of Nigeria, their production process, the health benefits associated and future trends of these foods.

2 Classification of Traditional Fermented Food Products of Nigeria

i Starch/Cassava-based Fermented Products

(a) Gari

Gari is a creamy-white, coarse flour with a slightly sour taste. It is a starch-based fermented food made from cassava tubers (*Manihot* sp.) and is popularly consumed in the South-Western and South-Eastern part of the country. *Gari* is produced by solid-state fermentation of cassava tubers. The cassava tubers are harvested, peeled, and washed. The cleaned tubers are grated and the pulp is transferred into polypropylene bags, which are then tied and hydraulic-pressed for 3–5 days at room temperature in order to allow fermentation by many desirable microorganisms. At this stage, the activity of LAB species gives characteristic flavor and sourness to the *gari*. Also, the cyanide compounds (cyanogenic glycoside, linamarin, and lotaustralin) present in the cassava, which may release toxic HCN to the food and cause food poisoning or lead to degenerative diseases, are detoxified (since larger amount of cassava varieties grown in Nigeria are high in cyanogens, which tend to be evenly distributed throughout the roots). After fermentation, the pulp is sieved and roasted with or without the addition of palm oil in a shallow cast-iron pan over an open fire with constant stirring with a piece of broken calabash for about 30 minutes to produce *gari* flakes. Figure 2.1 shows the flow chart of *gari* production. *Gari* is consumed either by soaking in cold water or as a solid when mixed with hot water (commonly known as *eba*).

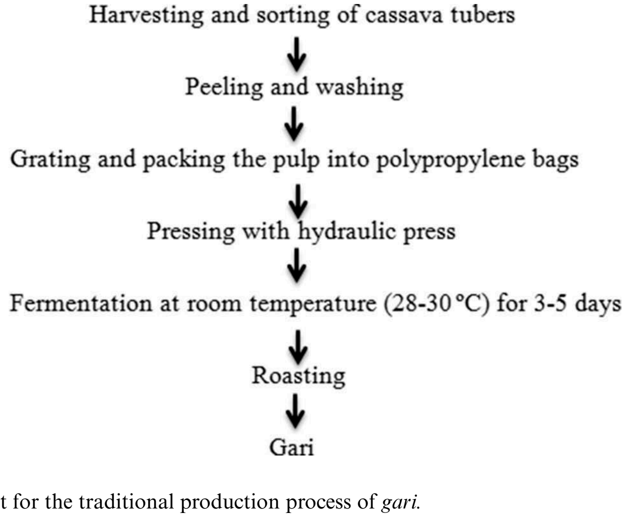
Harvesting and sorting of cassava tubers
Peeling and washing
Grating and packing the pulp into polypropylene bags
Pressing with hydraulic press
Fermentation at room temperature (28-30 °C) for 3-5 days
Roasting
Gari

FIGURE 2.1 Flowchart for the traditional production process of *gari*.

(b) Fufu

Fufu is another popular starch-based food produced from fermented cassava tuber. It serves as a staple food among the populace, especially the South-Eastern part of the country where it is popularly called *akpu* and among the South-Western dwellers. *Fufu* is fermented white slurry cooked into a thick paste. It is consumed with different kinds of indigenous soups. The method of production is by submerged fermentation, and the production process entails harvesting the cassava tubers, peeling, cutting the tubers into cubes, steeped in water, and allowing fermentation to take place for 3–4 days, thereby softening the root. At this stage, the cyanogenic compounds are removed via dewatering. The process is also characterized with offensive aroma produced by the activity of LAB during fermentation. The roots are mashed with hand to a soft gel, sieved, and then allowed to settle down. The resulting slurry is then cooked to form a thick paste.

(c) Lafun

Lafun is a starch-based food prepared from fermented cassava tuber. Processed *lafun* is in powder form and is white in color. *Lafun* also undergoes submerged fermentation, which is similar to the *fufu* production process. First, the cassava tubers are harvested, peeled, cleaned, and cut into cubes, and then steeped in water to allow fermentation for 2–3 days, after which they are sun-dried and milled into fine flour. The flour is made into dough by adding it into boiling water and stirring. *Lafun* is popular and widely consumed in the South-Western part of the country (among the Yoruba's). It is commonly eaten with *gbegiri* and *ewedu* soup (indigenous soup popularly consumed among the Oyo tribe, South-West Nigeria). The flowchart for the production process is shown in Figure 2.3.

(d) Abacha

Abacha is a popular snack food produced from cassava tuber. It is common among South-Eastern Nigeria. The production process of *abacha* involves harvesting and peeling the cassava tuber, which is then boiled in water for about 1 hr and cut into slices, then steeped in water for 1–2 days, changing water twice in order to remove any cyanogens that may be present, then it is finally washed two to three times and consumed with coconut or palm kernel/ groundnuts.

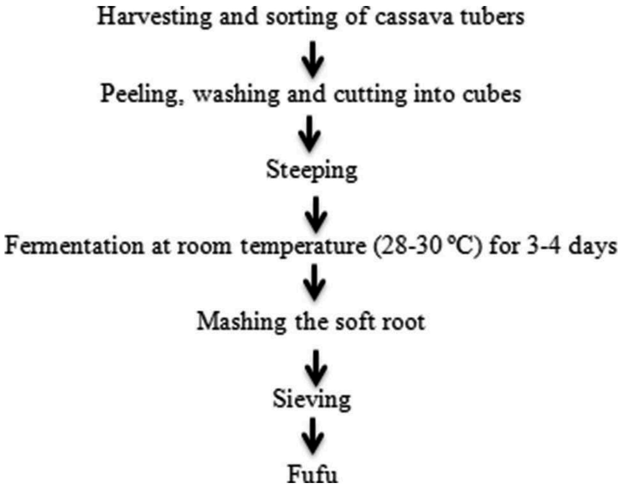
Harvesting and sorting of cassava tubers
Peeling, washing and cutting into cubes
Steeping
Fermentation at room temperature (28-30 °C) for 3-4 days
Mashing the soft root
Sieving
Fufu

FIGURE 2.2 Flowchart for the traditional production process of *fufu*.

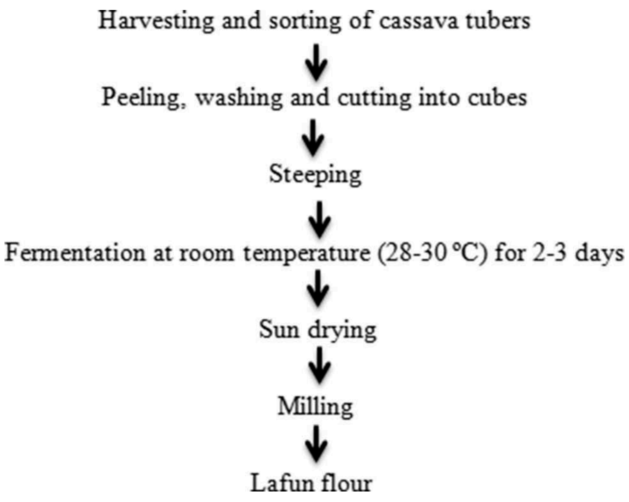
Harvesting and sorting of cassava tubers
Peeling, washing and cutting into cubes
Steeping
Fermentation at room temperature (28-30 °C) for 2-3 days
Sun drying
Milling
Lafun flour

FIGURE 2.3 Flowchart for the traditional production process of *lafun*.

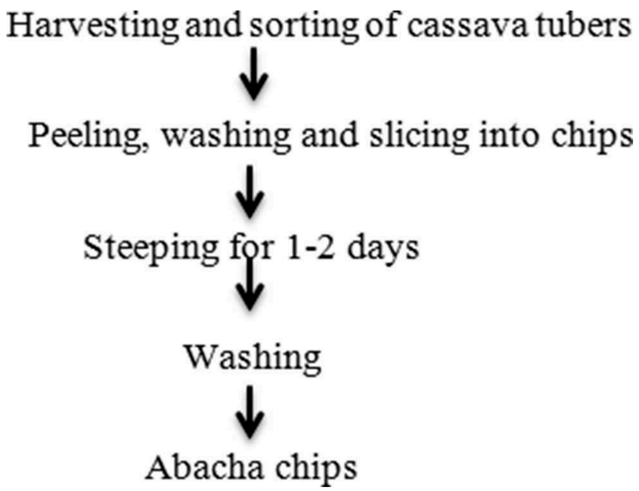
Harvesting and sorting of cassava tubers
Peeling, washing and slicing into chips
Steeping for 1-2 days
Washing
Abacha chips

FIGURE 2.4 Flowchart for the traditional production process of *abacha*.

ii Cereal-based Fermented Products

(a) Ogi

Ogi is popularly referred to as *akamu* or *koko* among the indigenous people. It is a mild acidic viscous gruel. It is used as weaning food and also consumed as a breakfast beverage or light meal with bean cake, milk, or sugar among the adults. *Ogi* can be produced from fermentation of different cereals such as maize (white variety; *ogi funfun* and yellow variety; *ogi pupa*), sorghum (red and white varieties; *ogi baba*), and millet (*ogi jero*). The production/fermentation process involves cleaning, washing, and steeping the cereal grains in clean water for 3–4 days (at this stage, the activities of the LAB and yeast present allow fermentation), after which they are milled and

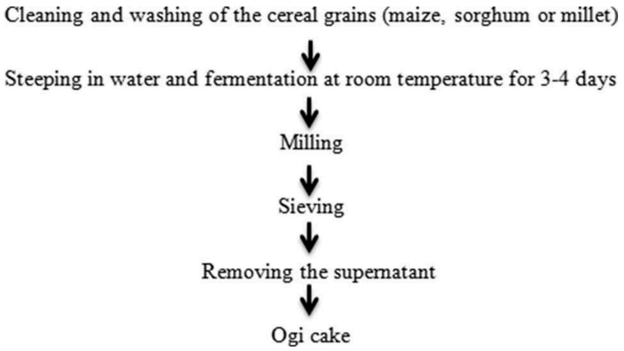
Cleaning and washing of the cereal grains (maize, sorghum or millet)
Steeping in water and fermentation at room temperature for 3-4 days
Milling
Sieving
Removing the supernatant
Ogi cake

FIGURE 2.5 Flowchart for the traditional production process of *ogi.*

sieved to remove the hull or any fibrous material that may be present. The cereal grains are then allowed to settle down and the supernatant is decanted (Figure 2.5). The *ogi* cake is reconstituted into a little cold water and poured into hot water to form a gruel after few minutes. *Ogi* is commonly used in the Southern States of Nigeria.

(b) Eko

Eko (also known as *agidi*) is a cereal-based fermented food commonly made from fermented maize (white and yellow varieties). The production process is similar to that of *ogi*, but with difference in the cooking method. The fermented *ogi* cake is cooked for a longer period to gelatinize and form a smooth paste. It is then wrapped in banana leaves and allowed to cool. It is usually consumed with different indigenous stew and soup. (See Figure 2.5 for the production process.)

(c) Masa

Masa (also known as *waina*) is commonly consumed by all age groups in the Northern part of Nigeria and some regions in the South-West. It is a traditional snack product usually taken with granulated sugar and is made from fermented maize or rice grains. It is served during ceremonies such as funerals, weddings, or during a child's dedication. The traditional production of *masa* involves steeping of the maize grain in warm water for about 6 hr, followed by wet-milling with little water and fermentation for about 24 hr. The fermented maize grains are then mixed with wheat flour in a 4:1 ratio (fermented maize grains: wheat flour) and ingredients, such as onions and salt, are added. The mixture is rolled to balls and fried in vegetable oil for a few minutes (Figure 2.6).

(d) Kunun-zaki

Kunun-zaki is a cereal-based nonalcoholic fermented beverage that is popularly consumed by all age groups in Northern Nigeria and some parts of Southern Nigeria. *Kunun-zaki* is served as a refreshing drink used for entertainments, appetizers, or during social gatherings because it is easily affordable. It is prepared from millet, maize, and sorghum grains, mostly with millet grain. The traditional production process involves steeping the millet grains for 2 days (at this stage, mainly LAB and yeasts are involved in the spontaneous fermentation process), wet-milling (addition of spices such as ginger, cloves, black pepper, and red pepper to improve taste and aroma), sieving, and gelatinizing with boiling water, then adding sugar to taste, followed by bottling (Figure 2.7).

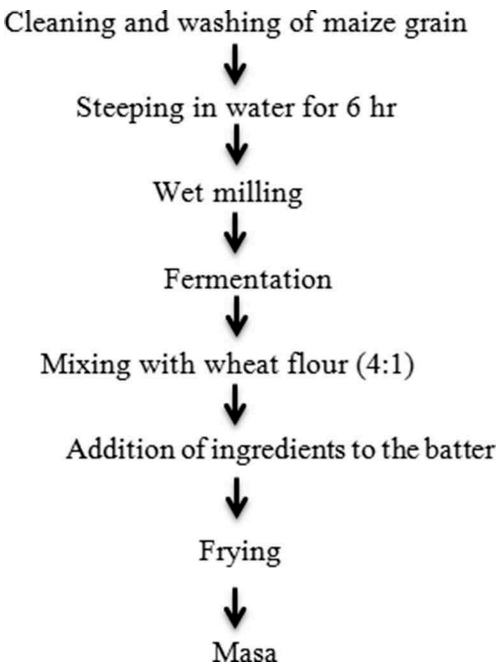
Cleaning and washing of maize grain
Steeping in water for 6 hr
Wet milling
Fermentation
Mixing with wheat flour (4:1)
Addition of ingredients to the batter
Frying
Masa

FIGURE 2.6 Flowchart for the traditional production process of *masa.*

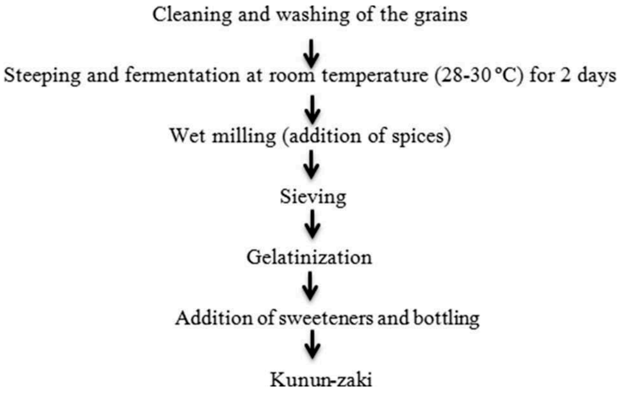
Cleaning and washing of the grains
Steeping and fermentation at room temperature (28-30 °C) for 2 days
Wet milling (addition of spices)
Sieving
Gelatinization
Addition of sweeteners and bottling
Kunun-zaki

FIGURE 2.7 Flowchart for the traditional production process of *Kunun-zaki.*

(e) Pito

Pito is a cereal-based fermented alcoholic beverage produced traditionally from the combination of maize and sorghum grains. It is a dark-brown liquid with a bitter taste. *Pito* is widely hawked among the middle-aged women in many Nigerian markets and is consumed by all age groups. The traditional production process involves soaking of the grains in water for 2 days, followed by malting for about 5 days (the process of germinating the grains, by allowing sprouting), after which the grains are wet-milled and then boiled for about 12 hr; it is allowed to cooled and filtered. The filtrate is allowed to stand and ferment overnight, after which the previously brewed *pito* is added (back slopping) and is again allowed to ferment overnight (Figure 2.8).

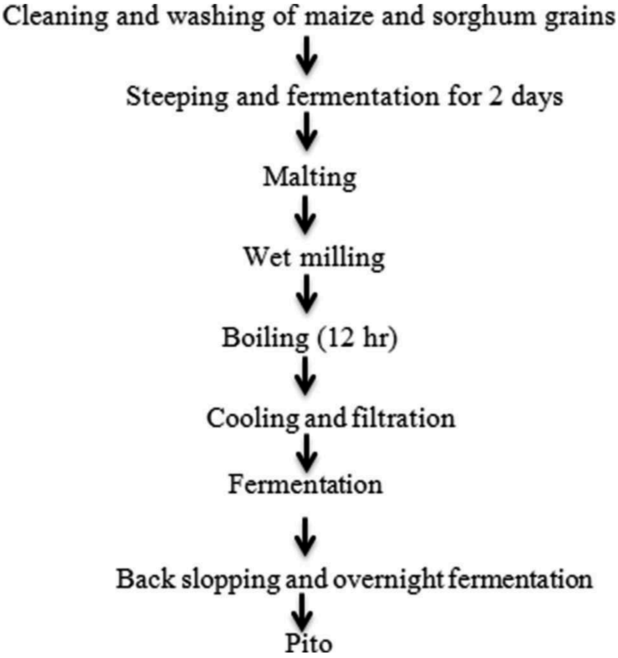
Cleaning and washing of maize and sorghum grains
Steeping and fermentation for 2 days
Malting
Wet milling
Boiling (12 hr)
Cooling and filtration
Fermentation
Back slopping and overnight fermentation
Pito

FIGURE 2.8 Flowchart for the traditional production process of *pito.*

(f) Burukutu

Burukutu is a fermented alcoholic beverage produced from malted sorghum. It is commonly consumed in the Northern part of Nigeria and also popularly sold in small markets attached to the Nigerian soldier barracks (Mammy markets). The production process includes steeping sorghum grains in water overnight, followed by malting, and allowing the grains to germinate for about 4–5 days. The malted grains are then sun-dried for 2 days, then ground to make powder. After which, *gari* (serves as an adjunct) and water are added to it, and the mixture is boiled for 12 hr (until properly cooked), filtered, and allowed to ferment for 48 hr (Figure 2.9).

(g) Sekete

Sekete is an alcoholic beverage produced from fermented maize grain. *Sekete* beer is popularly consumed by the natives of the western part of Nigeria. This beer is processed in a similar way as other fermented alcoholic beverages.

iii Dairy-based Fermented Products

(a) Nono

Nono is a dairy-based fermented beverage derived from cow milk and is traditionally referred to as *fura de nunu* because it is usually taken with sugar and *fura* (made from cooked millet flour compressed into balls). It has a sharp acidic taste and is widely consumed in the northern part of Nigeria, where the milking of the cow is majorly done by the Fulani herdsmen. The milking process is done between the third and sixth months of lactating cows, and it involves inoculating the fresh milk with a starter (leftover milk) and allowing fermentation for 24 hr at room temperature (Figure 2.10).

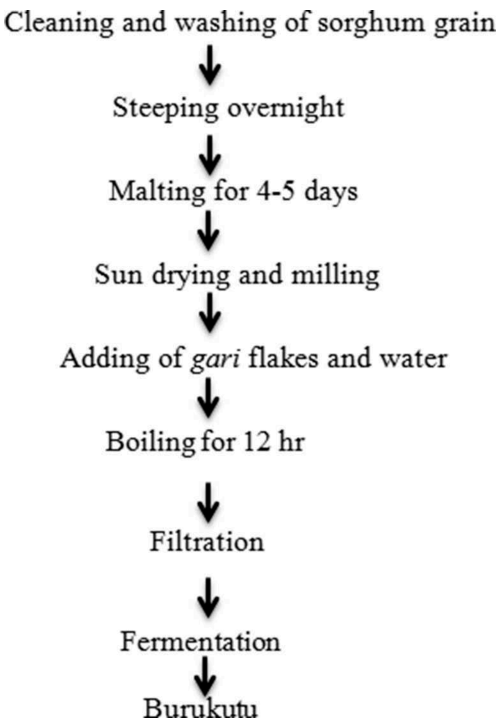

FIGURE 2.9 Flowchart for the traditional production process of *Burukutu* beer.

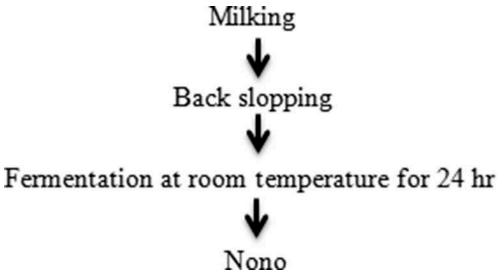

FIGURE 2.10 Flowchart for the traditional production process of *nono*.

(b) Wara

Wara is a traditional local cheese obtained from fermented cow milk. The traditional production process of *wara* involves crushing of Sodom apple leaves (*Calotropis procera*) to obtain juice, mixing the juice with cow milk, followed by mild heating over a fire. The milk then coagulates and the curd is allowed to drain, forming *wara* cheese. The production process is mainly carried out by the Northern dwellers (Figure 2.11).

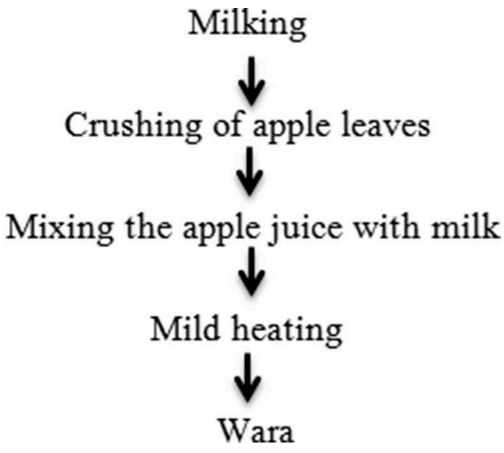

FIGURE 2.11 Flowchart for the traditional production process of *wara* cheese.

iv Vegetable/Seed-based Fermented Condiments

(a) Iru

Iru (also known as *dawadawa*) is an important fermented condiment produced from African locust bean (*Parkia biglobosa*) seeds. This indigenous condiment is added to different types of soups in Nigeria and consumed by different tribes in Nigeria. It gives the food a pleasant taste and appealing flavour; sometimes, it used as a substitute for meat or fish, especially among the low-income earners. The traditional production process involves boiling of African locust bean seeds for several hours until they are tender, followed by crushing underfoot to separate the hulls from the bean seeds (dehulling), then boiled for about 2 hr. During this boiling stage, softening agent(s) such as *kuru* or *kaun* is added to aid softening of the cotyledon, after which the bean seeds are packed in clean containers and allowed to ferment for 2–3 days at room temperature. Afterward, they are molded into small balls or flattened cakes (Figure 2.12).

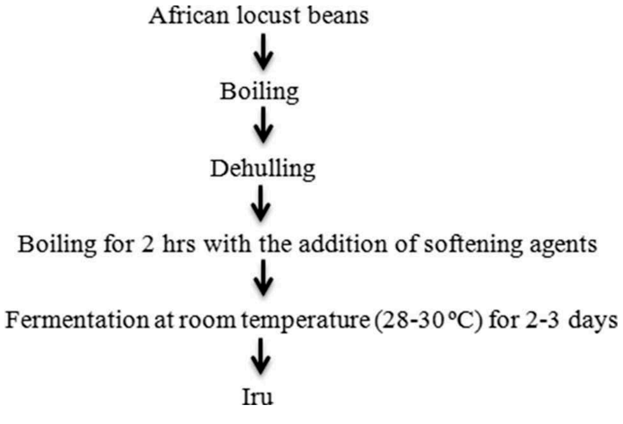
African locust beans
Boiling
Dehulling
Boiling for 2 hrs with the addition of softening agents
Fermentation at room temperature (28-30 °C) for 2-3 days
Iru

FIGURE 2.12 Flowchart for the traditional production process of *Iru.*

(b) Okpehe, Ogiri, and Ugba

Other indigenous fermented condiments produced from different seeds/vegetables and flowering plants include *okpehe* from the seed of flowering plant belonging to genus *Prosopis* (*Prosopis africana* seeds), *ogiri* from watermelon seeds (*Citrullus vulgaris*) or castor bean seeds (*Ricinus communis*), and *ugba* from seeds of African oilbean tree (*Pentaclethra macrophylla*). These fermented condiments are traditionally produced in a similar way as *iru* (as described above), but the duration of their initial boiling stage varies, with no addition of softening agents.

v Other Traditional Fermented Plant-based Products

(a) Emu

Emu (palm wine) is a refreshing mildly alcoholic beverage produced from palm sap. It is whitish in color. It is popularly consumed in Nigeria and in some parts of the West African countries. The production process involves tapping of palm sap from palm tree overnight into a gourd. It is then allowed to ferment for a few hours, after which it is ready for consumption. *Emu* is served during traditional marriage ceremonies or village meetings/gatherings at village squares.

(b) Agadagidi

Agadagidi is an indigenous beverage that is popular in the South-Western part of Nigeria. It is produced from the fermentation of plantains/banana. The production of *agadagidi* involves peeling, slicing, and steeping of plantain in water for about 3–5 days, after which the juice is collected and used as a drink.

3 Health Benefits Associated with Fermented Foods

In Nigeria, consumption of indigenous fermented foods has increased greatly because of the numerous health benefits associated with it. Fermented foods are considered natural and healthy because they contain live cells of desirable microorganisms and have been consumed for over thousands of years without any harmful effect. These foods are easily affordable and do not require any sophisticated equipment or machine for their production. During fermentation processes, the microflora present in the raw materials convert the chemical composition in the food, thus increasing its nutritional value and also imparting therapeutic effects to the consumer (Steinkraus, 2002; Tamang and Kailasapathy, 2010; Tamang, 2015).

Fermented cereals are often used as weaning foods by many because they are inexpensive to produce and also contain high nutritional contents when compared with the raw substrates (Nwachukwu et al., 2010). These foods are easily digestible, and their consumption confers a beneficial effect on human and reduces the risk of having contaminated foods, as most pathogenic organisms cannot survive during lactic acid fermentation process because of low pH (Chelule et al., 2010). Thus, fermentation improves the final food texture, organoleptic properties, nutritional value, and food digestibility. Slurries of these fermented cereals have been reported to exhibit health-promoting properties such as control of gastroenteritis and alleviating the incidence of diarrheal cases among the infants and older people (Olukoya et al., 2011)

Fermented foods have a prolonged shelf life, which makes them safe for consumers in terms of stability, transportation, and storage. Also, the LAB present during the fermentation processes display antimicrobial activity against food spoilage and foodborne pathogens (Russo et al., 2017). These foods have also increased the bioavailability of minerals such as calcium, phosphorus, zinc, iron, and amino acids because during the fermentation processes, the antinutrient factors such as phytic acid and tannins are decreased (Hemalatha et al., 2007; Murwan and Ali, 2011).

The activities of microorganisms remove toxic compounds and degrade mycotoxins that may be present in raw substrates during fermentation, without altering the nutritional quality of the food (Adedeji et al., 2017; Ari et al., 2012). Fermented foods also act as deterrents to pathogenic organisms because their consumption modifies the intestinal composition and restores the balance of gut bacteria, which reduces abdominal cramps, allergies, and lactose intolerance in patients with impaired immune system (Abdel et al., 2009; Olukoya et al., 2011).

4 Future Trends of Nigerian Indigenous Fermented Foods

The significance of fermented foods produced in Nigeria and Africa cannot be overemphasized. Therefore, measures should be taken to encourage their large-scale production, which will give room for industrialization of food products, thus boosting the nation's economy, as some of these indigenous fermented foods are not well known because they are produced on a small scale. Application of modern microbial biotechnologies will help in understanding the biochemical changes that occur during the fermentation processes. It will also offer a solution to the products with inconsistent quality because the microbes involved are tailored to perform the function of fermentation and to also mimic the microbial diversity usually associated with spontaneous fermentation (Holzapfel, 2002).

There are several research articles published on the microorganisms associated with spontaneous fermentation of indigenous foods and beverages (Odunfa and Oyewole, 1997; Oyewole et al., 2004; Olasupo et al., 2010; Adesulu-Dahunsi et al., 2018c, 2018d, 2018e). Also, several ongoing research activities focus on the identification and characterization of these autochthonous beneficial microorganisms isolated at different fermentation stages. All of these will aid in the implementation of starter culture technology for selection of appropriate strains with desirable probiotic, technological, and functional properties under controlled conditions, which are essential to obtain fermented food products safely and with consistent quality because sometimes there may be risks associated with spontaneous fermentation, such as production of food in unhygienic condition, involvement of undesirable microorganisms, and the drudgery encountered during the traditional production process. These technologies will increase the knowledge of food microbiologists/human nutritionists on the behavior and kinetics of beneficial microorganisms involved during the fermentation process and will also be useful in providing information pertaining to the designing of sophisticated fermentation tools and equipment (Achi, 2005; FAO, 2010).

Optimization of production/processing techniques by carefully manipulating process conditions such as inoculum, pH, temperature, quality and sizes of the substrate, hygienic handling practices, and the use of mechanized equipment for the production of traditional fermented foods and beverages produced in Africa will guarantee safety and improved shelf life. These will also increase the quantity of foods produced, thereby addressing food scarcity menace among the growing population, although several efforts have been made by the researchers in the past to address the provision of food for large growing populations and exportation of these foods, for example, packaged *ogi*, cassava flour, chips, flakes (*gari*) (produced by Federal Institute of Industrial Research, FIIRO, Oshodi), *dawadawa* cubes (Cadbury Nigeria Plc), bottled palm wine, and palm oil (Nigerian Institute for Oil Palm Research, NIFOR, Benin) (Iwuoha and Eke, 1996).

Few researchers have applied the combined method for examining diversity and safety of indigenous microflora that is associated with the traditional fermented foods and beverages. The combined use of culture-dependent and culture-independent methods of microbial identification and characterization, which are spontaneously present in the indigenous fermented foods, can be employed in safety standard compliance of dominant strains, as some microorganisms are nonculturable and may contain essential properties, thus playing important roles during the fermentation process (Oguntoyinbo et al., 2011; Greppi et al., 2013; Tamang et al., 2016). With the application of polyphasic approach, the dynamics, diversity, and roles of beneficial microorganisms involved in the fermentation of the indigenous fermented foods can be properly understood, also proper microbial identification will assist in the development of traditional fermented foods with probiotic features for improved health of the masses (Anukam and Reid, 2009; Adesulu-Dahunsi, 2018).

5 Conclusion

In Nigeria, fermented foods play a major role in the diet, the socioeconomic and cultural activities of the people. Up till date, the production process involves mainly the activities of microorganisms (LAB, yeast, and *Bacillus* sp.) and preparation remains a household art. Therefore, a comprehensive understanding of the diversity of microorganisms involved during the fermentation process and their production on a large scale will increase the popularity and acceptance even beyond the regions of Africa.

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Ethnic Fermented Foods of America

Cíntia Lacerda Ramos
Department of Basic Science, Federal University of Vales do Jequitinhonha e Mucuri, Minas Gerais, Brazil

Rosane Freitas Schwan
Biology Department, Federal University of Lavras, Lavras, Minas Gerais, Brazil

1 Traditional Fermented Foods and Beverages

Fermentation is one of the oldest forms of food preservation technologies in the world. It is a desirable process of biochemical modification of raw material performed by microorganisms and their enzymes, and fermented foods and beverages make up an important contribution to human diets in many countries. Among the fermented beverages, wine is cited in the Bible and in ancient Greek and Latin literature; beer was already known in ancient Egypt; and cider is probably as old as the other two cited beverages. Traditional fermented foods and beverages are produced and consumed by people from all parts of the world since ancient times. In many human communities, including indigenous people from America, they are still part of the traditional knowledge and are produced commonly on a small scale as a household or artisanal activity, particularly where—or when—agricultural surpluses are available (WHO 2014). Generally, the production of fermented food and beverages with high nutritional value is of medical and religious significance. In many cultures, the preparation and consumption of traditional alcoholic beverages is an occasional activity shared by people within the communities, often associated with festivals, religious rituals, or other special occasions (WHO 2014). American traditional fermented food products are homemade and produced at local and family level. Several substrates such as starch-rich maize, rice, and cassava are used for preparing fermented food. The type of raw materials and methodologies used for fermentation are often related to geographically isolated cultures. Alcoholic and nonalcoholic products are obtained by different methods of production. In general, long time of fermentation (more than 3 days) produces alcoholic beverages while short time (less than 3 days) is related to the production of nonalcoholic foods and beverages. A co-metabolism of bacteria, yeasts, and filamentous fungi is involved in ethnic fermented foods and beverages. Amongst the bacteria, lactic acid bacteria (LAB) species are highlighted due to their acid tolerance and ability to transport and metabolize different carbohydrates producing lactic acid, which is related to flavor and safety of the foods. Yeasts are mainly associated with the formation of alcohols (including ethanol in alcoholic beverages) and other aroma compounds, stimulation of LAB growth, improvement of nutritional values, and digestibility of the raw products. Although yeasts, especially *Saccharomyces cerevisiae*, are predominant microorganisms during alcoholic fermentation, bacteria species, such as *Lactobacillus* spp. and *Bacillus* spp. are also identified (Puerari et al. 2015; Santos et al. 2012).

Starchy substrates, such as maize, rice, and cassava, are frequently employed to prepare indigenous fermented foods and beverages. A common practice to incorporate amylase in the fermentation substrate to convert starch to fermentable sugars is the chewing corn or sweet potato and adding this mixture in the fermentation media as an inoculum. This practice is used by indigenous people from America to produce *chicha*, *calugi*, and *cauim* (Almeida et al. 2007; Elizaquível et al. 2015; Miguel et al. 2012; Puerari et al. 2015; Vallejo et al. 2013) to introduce the enzyme to break down the starch. Amylase converts the starch to glucose/maltose, which in turn is converted into ethanol by alcoholic yeast (e.g., *S. cerevisiae* and *Hansenula anomala*) or organic acids by LAB.

Ethnic fermented foods and beverages using starch-rich substrates in America have been described in several studies (Almeida et al. 2007; Chaves-López et al. 2014; Escalante et al. 2016; Miguel et al. 2012; Puerari et al. 2015; Ramos et al. 2010, 2011, 2015; Ray and Sivakumar 2009; Santos et al. 2012; Schwan et al. 2016). Cassava, rice, and maize are the most common substrates used by different ethnic groups in the world including American people to prepare a diversity of fermented foods and beverages, such as *calugi, cauim*, *yakupa*, *caxiri*, *chicha*, *parakari*, and *tarubá*.

Calugi is a fermented nonalcoholic porridge prepared from maize and rice by Javae Indians from Brazil. It is considered as daily basic diet that is consumed by adults and children. For preparation, flour obtained from dried maize and rice is diluted in water and cooked. An inoculum coming from mastication the sweet potato by Indians is also used for the preparation of the inoculated *cauins*. The mixture is fermented at room temperature (approximately 30 °C) (Miguel et al. 2012). Studies have shown the presence of *Lactobacillus plantarum, Streptococcus salivarius, Streptococcus parasanguis, Weissella confusa, Enterobacter cloacae, Bacillus cereus*, and other *Bacillus* spp., as well as the yeasts *S. cerevisiae, Pichia fermentans*, and *Candida* sp. A drop in pH from 6.0 to 3.5 after 48 h of fermentation was observed (Miguel et al. 2012), which guarantees the microbiological safety of the final product (Blandino et al. 2003).

Cauim is a nonalcoholic fermented beverage produced by different indigenous tribes in America from various substrates, such as rice, cassava, maize, peanuts, cottonseed, banana, pumpkin, and other fermentable fruits. The beverage is produced by traditional process and may be called ‘cooked *cauim*’ and ‘fermented *cauim*’. For preparation of cooked *cauim*, the substrates are cooked for around 2 h and cooled at room temperature. When the beverage gets cold, an inoculum may be added to initiate the fermentation process to prepare fermented *cauim*. The inoculum is obtained from the fluid generated by chewing sweet potato (Ramos et al. 2010; Schwan et al. 2007), as described for *calugi*. Both beverages are consumed by Indians, especially by children. Studies have shown that there is a predominance of LAB (*Lactobacillus* genus) in these fermentations, which confers the slightly acidic flavor triggered by the presence of lactic acid (Almeida et al. 2007; Ramos et al. 2010, 2011). Species of *Candida*, *Pichia*, *Clavispora*, *Kluyveromyces*, and *Saccharomyces* genera are the yeasts described in the fermentation of *cauim* prepared from different substrates, such as cassava, peanuts, and cottonseeds (Ramos et al. 2010, 2011; Schwan et al. 2007).

Yakupa is an indigenous nonalcoholic fermented beverage, produced by Indians from Brazil, consumed daily by children and adults and is within the category of *cauins*. To prepare the *yakupa*, cassava roots are first soaked in water for 2–3 days, this step allows prefermentation (detoxification) of the substrate. The prefermented roots, called puba, are dehydrated and crushed. Water is added to this mixture, which is then sifted with a sieve obtaining a white liquid which is cooked and mixed with grated sweet potatoes (Freire et al. 2014). The beverage is consumed as soon as it is prepared, as observed for cooked *cauim*, or the beverage is fermented for 24–48 h, as performed for fermented *cauim*. The fermentation of yakupa is dominated by heterolactic LAB species, mainly *L. fermentum* in addition to *L. plantarum*, *W. cibaria*, and *W. confuse*. The yeasts *S. cerevisiae* and *Pichia kudriavzevii* were also detected (Freire et al. 2014).

Chicha is an alcoholic fermented maize product widely consumed by indigenous groups prevalent from the Amazon to the Andes (Blandino et al. 2003; Chaves-López et al. 2014). Its preparation is a unique fermentation process, in which the fermentation of maize occurs for roughly 8 days. Saliva serves as the source of amylase for the conversion of starch to fermentable sugars, as already described for *calugi* and *cauim*. After fermentation, the resultant mass is diluted in water and filtered, and the liquid obtained is the beverage, which is ready to consume. Yeasts,

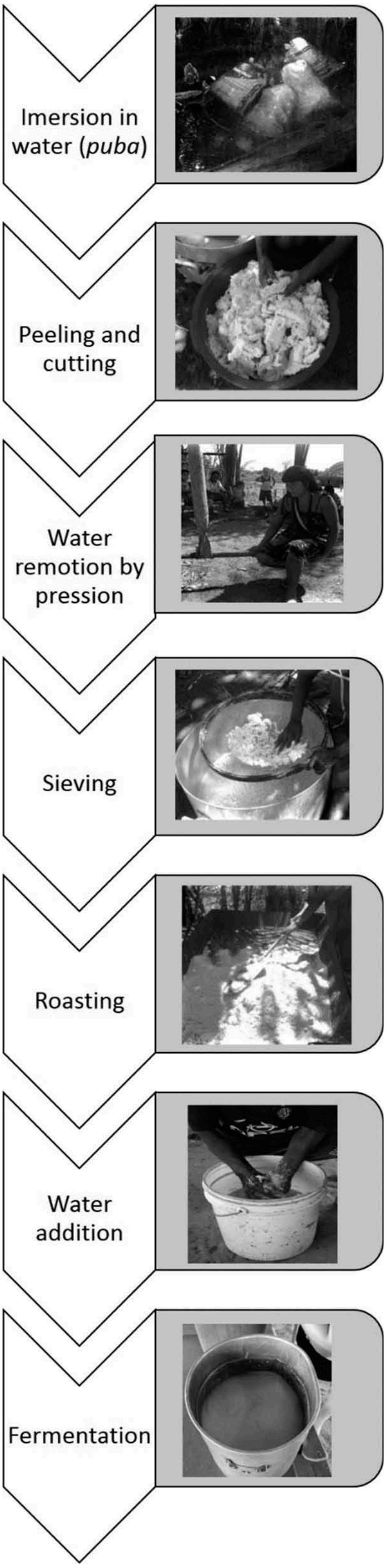

FIGURE 3.1 Flow diagram summarizing the preparation of the ethnic alcoholic beverage *caxiri.*

particularly *S. cerevisiae*, and bacteria of the genus *Lactobacillus* spp., *Leuconostoc* spp., and *Acetobacter* spp. with various fungi such as *Aspergillus* spp. are the primary fermenting microorganisms in *chicha*. The alcoholic content is generally low, but this can vary between 1 and 12% (v/v) depending on the production method such as substrate and time of fermentation (Chaves-López et al. 2014; Puerari et al. 2015).

Caxiri is an ancient alcoholic fermented beverage produced from cassava and consumed by many indigenous tribes from the Amazonian region. Its alcohol content is about 10/11° GL and in addition to cassava, sweet potato, yam, and/or sugarcane juice can be added to prepare the beverage. In Brazil, to prepare the *caxiri*, the Juruna tribe starts by immersing cassava roots in running water for 2 days to produce a softened and fermented product, the *puba* (Santos et al. 2012). After the retting of cassava, the tubers are peeled, cut into small pieces, pressed to remove excess water, grated, sieved, and roasted to produce flour. The roasting process is important because it contributes significantly to the detoxification of cassava roots (Montagnac and Tanumihardjo 2009). The flour is then diluted with water, mixed with grated sweet potatoes and placed into barrels to initiate the fermentation process. The mixture is kept in the barrels for about 24–48 h at approximately 30 °C. The beverage is consumed within 48 h after preparation (Santos et al. 2012). A flow diagram representing *caxiri* preparation is shown in Figure 3.1. Santos et al. (2012) found that *S. cerevisiae* was the predominant yeast species while for bacteria there was a major involvement of spore-forming bacteria belonging to the genus *Bacillus*. *Caxiri* may also be produced using a blend of cassava and corn, showing a predominance of *Saccharomyces cerevisae*, *Rhodotorula mucilaginosa*, *Lactobacillus fermentum*, *Bacillus subtilis*, and *Lactobacillus helveticus* (Miguel et al. 2015).

Tarubá is another beverage produced from cassava by Indians living in the Brazilian state of Amazonas. Its preparation uses solid substrate fermentation, unlike the submerged fermentation employed for other cassava beverages. After solid-state fermentation, the dough is diluted in water for consumption. To prepare the beverage, cassava roots (*Manihot utilissima*) are peeled and washed in running water. Then, the cassava is crushed, pressed, and sieved to remove excess water using a handmade straw press called a 'tipiti'. The obtained mass is toasted and covered with leaves of *Trema micrantha* (L.) *Blume* and *Musa* spp. (banana) and is left to ferment for 12 days. After fermentation, the cassava dough is diluted in water and filtered to remove the solids. The liquid obtained is the beverage ready to be consumed (Ramos et al. 2015). Microbial studies on this beverage have also shown an association of bacteria (*Lactobacillus* spp. and *Bacillus* spp.) and yeast (*Torulaspora delbrueckii*) (Ramos et al. 2015).

In Guyana, a unique cassava beverage produced by Amerindians is *parakari*. This is unique because it involves the use of an amylolytic mold (*Rhizopus* spp.) followed by a solid substrate ethanol fermentation. This dual fermentation is similar to those fermentations performed in Asia for the production of alcoholic beverages from rice such as *sake* and different than all those alcoholic beverages described in Latin America (Henkel 2005). The Amerindians use specific cassava varieties, control of culture temperature and boosting of *Rhizopus* inoculum potential with purified starch additives.

2 Starch-rich Substrates for Preparation of Ethnic Foods and Beverages

Maize, rice, and cassava are important substrates cultured and used as substrate by human communities for production of a diversity of fermented foods and beverages in different parts of the world. Maize is, after wheat and rice, the most important cereal grain in the world, providing nutrients for humans and animals. It serves as a basic raw material to produce alcoholic beverages and other food products including starch, oil, protein, and food sweeteners. Maize grain accounts for about 15–60% of the total daily calories in diets of people in developing countries, particularly in Africa and Latin America. Many of the traditional foods are produced from fermented or geminated maize, which increases the vitamin content, mineral bioavailability, and the quality of protein. Like other cereals, maize is an excellent source of carbohydrates, which accounts for about 72–73% of the kernel. Maize also contains protein, but it is regarded as inferior because of its low content of lysine and tryptophan. It is considered to be rich in vitamin B and minerals, especially, phosphorus, magnesium, manganese, zinc, iron, and small amounts of potassium. Maize is deficient in niacin, which is found in the outer layer and often removed with the pericarp during dehulling. Maize compares well with other cereals as energy source. However, maize contains no tannins as compared to some sorghum and finger millet cultivars, substrates that are also used for the production of fermented beverages.

Rice is known as the grain of life. It is one of the most important food crops in terms of area, production, and consumer preference. Rice is an important substrate for indigenous fermented foods, not only in Asian countries, but also in America. It contains 80% carbohydrates, 7–8% protein, 3% fat, and 3% fiber. The mineral content, starch quality, glycemic index, and antioxidant activity has made rice unique among cereals. Rice is also consumed in different forms such as flour, paste, boiled, flattened, fried, dried, and as sprouted seedlings. The fermentation enriches the properties of rice, supplements it with different essential amino acids, vitamins, minerals, prebiotics, probiotic organisms, and degrades antinutrients (phytic acid, tannins, and polyphenols) (Freire et al. 2017a).

Cassava (*Manihot esculenta* Crantz) plays an important role in the global food diet, mainly in developing countries; however, it is considered an inferior food, as the roots are low in protein, essential minerals, and vitamins and it is highly perishable (roots get spoiled within 48–72 h after harvest) (Vasconceloset al. 1990). Furthermore, certain varieties of cassava contain large amounts of cyanogenic glucosides (linamarin and lotaustralin), which are toxic to humans and mammals; additionally, they are not palatable. Fermentation is an important means of processing cassava to improve its palatability, textural quality, and to upgrade its nutritive value by enrichment with proteins and for reduction of cyanogenic glucosides (Mkpong et al. 1990). Fermentation process reduces the cyanide level from 10–49 mg HCN equivalent kg^{-1} raw cassava to 5.4–29 mg HCN equivalent kg^{-1} in several fermented products (Edijala et al. 1999; Vasconcelos et al. 1990), which is well below the safe level of 30 mg HCN equivalent kg^{-1} recommended by Almazan (1986). Other benefits accrued from cassava fermentation are longer shelf life (3–30 days) of the fermented food products in comparison to that of fresh roots (48–72 h), improved palatability and textural qualities, nutritional enhancement, functional and probiotic attributes, and ease of processing at home and cottage-industry levels.

2.1 Processing for Cassava Detoxification

Cyanide detoxification by fermentation and linamarase action has been reported (Lei et al. 1999; Westby and Choo 1994). Fermentation allowed the elimination of more than 90% of endogenous cyanide compounds in the roots. The elimination mostly occurred after 48 h, when the endogenous cassava linamarase reached its optimum pH 5.5 (Ampe and Brauman 1995). LAB linamarase may participate in the cyanogen degradation, and the bacterial pectinases have also been shown to help the process (Ampe and Brauman 1995). According to Santos et al. (2012), for *caxiri* production by Brazilian Amerindians, bitter cassava roots are fermented for 2 days in running water to soften the skin. This softened and fermented cassava is known as *puba*. The 'pubagem' process is a fast and easy solution for toxicity elimination of bitter cassava roots. In addition, it allows uniform softening of the plant tissue. This process consists of washing cassava, cutting them into pieces, and submerging them in water for a period of 3–7 days at room temperature. They may be immersed in large pots with fresh water or can be placed in bags of linen and left on the rivers. The removal of cyanogens from cassava during semi-solid fermentation, as 'pubagem', is probably the result of several factors including (1) textural changes in the plant tissues that make it possible for vacuole-bound CGs to diffuse, and come in contact with membrane-bound linamarase and for hydrolyzed and intact compounds to leach out; (2) increase in β-glucosidase activity in cassava tissue; and (3) utilization of CGs and their breakdown products by fermentation of microorganisms (Onabolu et al. 1999).

3 Microorganisms in Ethnic Fermented Beverages

Fermented beverages typically involve a variety of microorganisms that contribute to sensorial characteristics of the product, as well as improve nutrient preservation and increased shelf life. As described above, different fermented beverages produced from maize, rice, cassava, and the associated microorganisms (e.g., bacteria, yeasts, and filamentous fungi) have been described. The microbial diversity related to fermented beverage has a direct effect on the sensorial, nutritional, and safety properties of the final product. It is directly related to the quality and safety of fermented beverage and may be associated with the source (e.g., substrate and process), as well as the fermentation conditions that provide a particular environment for the growth of the microbial communities. The indigenous fermented beverages are generally prepared based on empirical knowledge transferred from generation to generation, thus the contaminant microorganisms may be also associated with the environment, the water, or the nonsterile utensils used to produce the food. The temperature, pH, acidity, alcohol (for alcoholic beverages), salt, nutrients, antimicrobial compounds, and competitive microorganisms, among other factors, are important environmental conditions for establishing microbial communities in indigenous fermented beverages.

Bacteria, yeasts, and filamentous fungi may be involved in these kinds of beverages. Filamentous fungi, such as *Aspergillus* spp., *Rhyzopus* spp., and *Mucor* spp. have important role in fermented beverages production by saccharification of starch present in the substrate (maize, rice, and cassava), although, several American fermented beverages employ saliva as an amylase inoculum. *S. cerevisiae* is the most frequent yeast isolated from different alcoholic indigenous fermented beverages. This yeast is mainly associated with ethanol production. In addition, other species, such as *Torulaspora delbrueckii, Candida* spp., *Pichia* spp., and *Kluyveromyces* spp., have been also found in maize, rice, and cassava alcoholic beverages. Yeasts are important producers of volatile compounds, which are associated with the sensorial characteristics of fermented products. Freire et al. (2017b) elaborate a nonalcoholic fermented beverage from cassava and rice and found that the co-culture of LAB with the yeast *T. delbrueckii* improved the product's digestibility by reducing starch, increasing the antioxidant activity, and further stimulating probiotic LAB growth during fermentation.

Among the bacteria groups, LAB and spore-forming bacteria *Bacillus* spp. have been widely described, and they play a very important role in many types of indigenous fermented foods and beverages. LAB constitute a group of Gram-positive, nonsporulating, aerotolerant bacteria that produce lactic acid as one of their main fermentation products. LAB species play very important roles in the production of a range of traditional fermented foods and beverages due to their acid tolerance and ability to transport and metabolize different carbohydrates. LAB not only enhance flavor and texture, but they also inhibit pathogenic and spoilage organisms by several mechanisms, such as the production of organic acids, hydrogen peroxide, and antimicrobial substances, as well as by lowering pH and redox potential. LAB frequently grow in association with yeasts, favoring their growth by lowering pH (Freire et al. 2017b). The fermenting yeasts may proliferate and spontaneously promote alcoholic fermentation. *Bacillus* are Gram-positive, aerobic, motile, spore-forming rods that produce catalase. *Bacillus* species are attractive industrial organisms for several reasons, including high growth rates resulting in short fermentation times, the capacity to secrete proteins, and high enzymatic activity. Although the role of *Bacillus* species in cassava-fermented products has not been reported in detail, Amoa-Awua and Jakobsen (1995) demonstrated the ability of some of these species (e.g., *B. subtilis* and *B. pumilus*) to hydrolyze the tissues of cassava through the production of cellulases. *Bacillus subtilis* have shown important properties in soybean fermentation by producing many enzymes, including amylases, cellulases, and proteases. The proteases are responsible for the main flavor, through hydrolysis of soybean protein (Joo et al. 2007; Schallmey et al. 2004). More than 90% of *Bacillus* species (e.g., *B. subtilis*, *B. amyloliquefaciens*, and *B. licheniforms*) isolated from the cassava beverage *taruba* displayed amylolytic and proteolytic activities (Ramos et al. 2015).

3.1 Yeast

Yeasts are often employed in industrial fermentation processes for their ability to efficiently convert sugars into ethanol and carbon dioxide. In addition, the fermenting yeasts produce a wide range of other compounds in alcoholic fermented beverages, including various higher alcohols, carbonyl compounds, phenolic compounds, fatty acid derivatives, and sulfur compounds. Figure 3.2 shows an overview of yeast metabolism (primary and secondary metabolites). Many of the secondary metabolites are volatile and have specific flavors that are associated to sensorial quality of the final beverage. The genus *Saccharomyces*, especially *S. cerevisiae*, is strongly associated with the production of fermented products for human consumption such as bread and the very known alcoholic beverages (e.g., wine and beer). This specie is also dominant in several traditional alcoholic fermented beverages produced from starch-rich substrates (Miguel et al. 2012, 2015; Santos et al. 2015; Schwan et al. 2007). During the years of yeast usage for different purposes, through phenotypic selection, the species evolved to produce goods with organoleptic properties pleasant to humans. In this sense, given the high degree of diversity found in nature, it is expected to find yeast with new and more interesting characteristics for fermented beverages and industry.

In many industrial fermentation processes, ethanol is the most important compound produced by yeast. Moreover, it is the production of this primary metabolite that originally sparked interest for the fermentation of beverages. Although glucose is commonly used as the sole carbon source for yeast growth in the laboratory, in the starch-rich substrates, such as maize, rice, and cassava, the main carbohydrates available for yeast metabolism are maltose, glucose, and maltotriose. The central metabolism begins with the basic conversion of carbohydrates into pyruvic acid, yielding energy in the form of ATP and reduced NADH cofactors. The sequence of enzyme-catalyzed reactions that convert glucose to pyruvic acid is known as glycolysis and can be summarized as:

$$Glucose + 2ADP + 2Pi + 2NAD^+ \rightarrow 2Pyruvate + 2ATP + 2NADH^+ + 2H^+$$

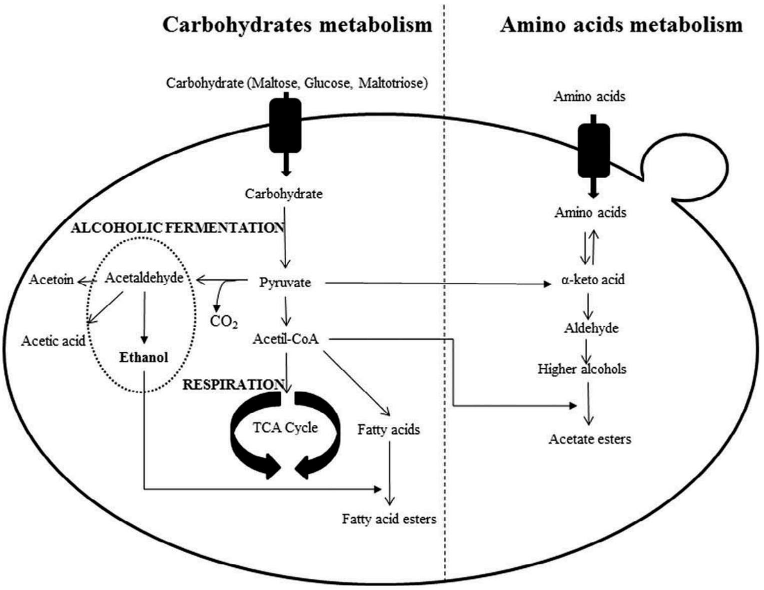

FIGURE 3.2 Pathway in yeasts: carbohydrate metabolism (fermentation and respiration) and amino-acid metabolism (aldehydes, higher alcohols, and ester formation).

The conversion of pyruvate to ethanol is a two-step process. First, pyruvate is converted to acetaldehyde by a pyruvate decarboxylase, releasing carbon dioxide. Then, acetaldehyde is subsequently converted into ethanol by an alcohol dehydrogenase:

$$CH_3COCOOH \rightarrow CH_3CHO + CO_2 \rightarrow CH_3CH_2OH$$

Overall, the ethanol fermentation is a redox-neutral process, since the reduced coenzyme NADH produced during glycolysis, in the oxidation of glyceraldehyde 3-phosphate, is latter re-oxidized in the reduction of acetaldehyde to ethanol:

$$2Pyruvate + 2NADH + 2H^+ \rightarrow 2NAD^+ + 2Ethanol + 2CO_2$$

Acetaldehyde can also be acted on by an aldehyde dehydrogenase to produce acetic acid:

$$CH_3CHO \rightarrow CH_3COOH$$

Additionally, an acetaldehyde molecule can interact with an additional acetaldehyde to form acetoin:

$$CH_3CHO + CH_3CHO \rightarrow C_4H_8O_2$$

The theoretical (stoichiometric) conversion of glucose to ethanol is as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Therefore, for each kilogram of glucose fermented, approximately 500 g of ethanol can theoretically be produced. However, during alcoholic fermentations, the best yields are only ~90% of the theoretical conversion equation due to some fermentable carbon being diverted to other by-products. Besides the direct products of fermentation, ethanol and CO_2, several other products are generated during the process, including cell biomass, glycerol, some organic acids, and minor fermentation metabolites (flavoring compounds, etc.).

3.1.1 Secondary Fermentation Metabolites Produced by Yeasts

Secondary fermentation metabolites of yeasts include higher alcohols, polyols, esters, organic acids, vicinal diketones, and aldehydes (Figure 3.2 and Table 3.1). These metabolites, although they are produced by yeast in much lower concentrations compared with ethanol and carbon dioxide, are very important for flavor characteristics in fermented beverages. Two of the main secondary fermentation metabolites are glycerol and succinic acid. Glycerol contributes to the viscosity of fermented beverages as wine (Walker 2014). After ethanol and CO_2, glycerol is the largest concentration metabolite. Succinic acid may be produced by *S. cerevisiae* following limited operation of the citric acid cycle (TCA cycle), whereas glycerol may be produced in redox-balancing reactions, or as a compatible solute in response to osmotic stress (Walker and Stewart 2016). Regarding esters, these compounds represent a very important group of flavor-active compounds that generally result in desirable fruity/floral flavors and aromas in fermented beverages. Most of these are produced by yeast during fermentation in reactions between alcohols and acyl-CoA molecules, but some esters, notably ethyl lactate, are linked to bacterial co-existence (e.g., from *Lactobacillus* spp.). The concentration of esters produced during fermentation depends on the relative abundance of the corresponding alcohols and acyl-CoAs, but since acetyl-CoA and ethanol are the most abundant, ethyl acetate is the predominant ester produced. Isoamyl acetate is produced in lower concentrations, but as it has a much lower flavor threshold than ethyl acetate, it is a more significant contributor to beverage aroma. Diacetyl is a vicinal diketone produced by *S. cerevisiae* during a side reaction in the synthesis of the amino acid valine. The presence of diacetyl in alcoholic fermented beverages is undesirable, as it imparts a rancid-butter flavor sometimes referred to being akin to butterscotch.

The production of higher alcohols or fusel oils during fermentation follows amino acid deamination and decarboxylation, known as the Ehrlich Pathway, a catabolic route that comes into play when there are excess amino acids in the medium. When these are deficient, an alternative anabolic route called the Biosynthesis Pathway becomes operational, deriving higher alcohols from α-keto acid intermediates (Dzialo et al. 2017). In general, higher temperatures during fermentation favor increased levels of fusel oils. These compounds are particularly important for flavor development in alcoholic beverages, and the levels of individual fusel oils in beverages are linked to the levels of corresponding amino acids in the fermentation medium (e.g., phenylalanine stimulates phenyl ethanol production leading to a rose-like aroma).

3.2 Bacteria

LAB and *Bacillus* spp. are the main bacteria groups isolated from different ethnic fermented foods and beverages in America. LAB is a Gram-positive bacteria group, nonsporulating, and aerotolerant that produce lactic acid as one of their main fermentation products. They belong to the phylum Firmicutes, class Bacilli, order Lactobacillales, and are included in different families such as Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, and Streptococcaceae (www.uniprot.org//taxonomy/186826). According to their carbohydrate metabolism, LAB are classified as: (i) obligatory homofermentative, which bacteria is able to metabolize hexoses via glycolysis only, yielding solely lactic acid; (ii) obligatory heterofermentative, which bacteria uses the phosphoketolase pathway that forms lactate and ethanol or acetate from hexoses and pentoses; and (iii) facultative heterofermentative, which bacteria degrades hexoses via glycolysis and pentoses through the phosphoketolase pathway.

Generally, LAB is the most prevalent bacteria group found in indigenous fermented foods and beverages. Their common occurrence in foods, in association to their long historical use, contributes to their acceptance as generally recognized as safe (GRAS) for human consumption (Silva et al. 2002). It is known that LAB contribute to food preservation and sensorial characteristics of the foods. In carbohydrate-rich environments, LAB frequently grow in association with yeasts, stimulating yeast growth (Freire et al. 2015). In addition, LAB are highlighted by their probiotic properties. Ramos et al. (2013) studied the probiotic characteristics of microorganisms isolated from different fermented foods and described a potential probiotic strain isolated from *cauim*, *L. brevis* UFLA FFC199. This isolate showed tolerance to low pH and bile salts, which are conditions imposed by the gastrointestinal environment. In addition, the isolate was able to adhere to a human intestinal epithelial cell line (Caco-2) and then increased the TEER (Trans-Epithelial Electrical Resistance) of the Caco-2 cell line.

The *Bacillus* genus belongs to Phylum Firmicutes; Class Bacilli; Order Bacillales; Family Bacillaceae (www.uniprot.org/taxonomy/1385). *Bacillus* spp. are Gram-positive bacteria, aerobic, motile, and spore-forming rods that produce catalase. Species from *Bacillus* genus have important industrial characteristics such as high growth rates in short fermentation times and the capacity to secrete proteins and enzymes. Moreover, some species, such as *B. subtilis* and *B. licheniformis*, are recognized as GRAS by the Food and Drug Administration. *Bacillus* species have been described in different indigenous fermented foods and beverages as already mentioned in this chapter (Miguel et al. 2012; Ramos et al. 2010, 2011, 2015; Santos et al. 2012); however, knowledge about their role in these fermentations is still limited. Some isolates of *Bacillus* identified by Ramos et al. (2015) were able to grow at 45 °C and showed amylolytic and proteolytic activities. They probably make an important contribution to the *tarubá* beverage and may be potential starter cultures for better control of the fermentation process.

TABLE 3.1

Volatile compounds detected in different traditional alcoholic fermented beverages and their aroma volatiles

Compound	Aroma volatiles	Associated beverage	Reference
Ethanol	Alcohol, fruity	Corn *chicha*	Resende et al. 2018
Furfuryl alcohol		Maize and cassava *caxiri*; cassava and corn *calugi*	Miguel et al. 2014, 2015
2-Phenylethanol	Roses	Cassava yakupa	Freire et al. 2014
3-Hexanol	Sweet, flower	Corn *chicha*	Resende et al. 2018
3-Heptanol	Alcohol	Corn *chicha*	Resende et al. 2018
Phenylethyl alcohol	Flowers, rose	Maize and cassava *caxiri*; corn *chicha*	Miguel et al. 2015; Resende et al. 2018
1,3-Butanediol		Cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014
Acetaldehyde		Maize and cassava *caxiri*; cassava *yakupa*; cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
1,1-Dietoxyethane		Cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014
Hexanal	Green, grass, pine	Cassava and corn *calugi*	Miguel et al. 2014
Octanal		Cassava and corn *calugi*	Miguel et al. 2014
Furfural		Maize and cassava *caxiri;* cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
Benzaldehyde	Vegetables, green	Corn *chicha*	Resende et al. 2018
Decylaldehyde		Cassava and corn *calugi*	Miguel et al. 2014
Diethyl malate		Maize and cassava *caxiri;* cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
Ethylacetate	Flowery, ester	Cassava and corn *calugi*	Miguel et al. 2014
Ethyl butyrate	Floral, fruity	Maize and cassava *caxiri*; cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
Ethyl acetate	Fruity	Maize and cassava *caxiri;* cassava *yakupa*	Freire et al. 2014; Miguel et al. 2015
Isobutyl acetate		Maize and cassava *caxiri*; cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
2-Phenylethyl acetate		Cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014
Acetic acid	Sour, vinegar	Corn *chicha*	Resende et al. 2018
Isobutyric acid	acidic, rancid	Corn *chicha*	Resende et al. 2018
Butyric acid	rancid, cheesy	Maize and cassava *caxiri*; cassava *yakupa;* cassava and corn *calugi;* corn *chicha*	Freire et al. 2014; Miguel et al. 2014, 2015; Resende et al. 2018
Pentanoic acid	Burnt rubber		
Propanedioic acid		Corn chicha	Resende et al. 2018
Hexanoic acid	Strong, sour, cheese	Cassava and corn *calugi;* corn *chicha*	Miguel et al. 2014; Resende et al. 2018
Octanoic acid	Urine-like	Maize and cassava *caxiri*; cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
Nonanoic acid	Roastnut, chemical	Maize and cassava *caxiri;* cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015
Decanoic acid		Maize and cassava *caxiri*; cassava *yakupa;* cassava and corn *calugi*	Freire et al. 2014; Miguel et al. 2014, 2015

(*Continued*)

TABLE 3.1 (Cont.)

Compound	Aroma volatiles	Associated beverage	Reference
Guaiacol	Chemical, hospital	Cassava and corn *calugi*	Miguel et al. 2014
a-Terpeniol		Cassava and corn *calugi*	Miguel et al. 2014
b-Citronellol		Cassava and corn *calugi*	Miguel et al. 2014
1,1-Diethoxyethane		Brazilian maize and cassava *caxiri*	Miguel et al. 2015

4 Starter Culture for Ethnic Fermented Foods and Beverages

A starter culture may be defined as a preparation or material containing large numbers of variable microorganisms, which may be added to accelerate a fermentation process. Being adapted to the substrate, a typical starter facilitates improved control of a fermentation process and predictability of its products. Starter cultures also facilitate control over the initial phase of a fermentation process (Holzapfel 2002).

Some traditional fermented foods use backslopping as starter culture. It is the inoculation of the raw material with a small amount of dough from a previous successful fermentation. Typical for this starter class are used in rice-based fermented beverage preparations and are mainly composed by fungi (*Amylomyces rouxii, Rhizopus* spp., and *Mucor* spp.) and yeasts (*S. cerevisiae, Hansenula* spp., *Endomycopsis filbuligera*, and *Candida* spp.) (Dung 2013; Dung et al. 2006).

Currently, studies and advances to improve the quality of foods and beverages industrially produced, such as cheese, yogurt, fermented milk, beer, wine, whisky, and others, have increased substantially. The search for adequate strains to initiate and dominate the fermentative process has gained attention of researchers from all over the world and commercial starter cultures are available in the market. On the other hand, there are no commercial starter cultures for indigenous fermented beverages. Although the literature about the chemical and microbial parameters of indigenous fermented foods produced in the world has increased in the last decades, very few advances were obtained in regards of industrial development. Most of the studies have described the typical way of preparation, the microbial diversity involved in the fermentation and the chemical parameters of the beverages (Blandino et al. 2003; Dung 2013; Kubo et al. 2014; Ramos and Schwan 2017). Little have been proposed about starter culture for traditional beverages in America (Freire et al. 2015, 2017a, 2017b). Freire et al. (2017b) evaluated different associations of LAB and yeasts to produce rice and cassava beverages and found that the association of the yeast *Torulaspora delbrueckii* and the LAB *L. plantarum* and *L. acidophilus* enhanced the aroma and provided safety of the product by lowering the pH. In addition, the using starter culture accelerated the fermentation process, reducing the risk of contamination, improved the product's digestibility by reducing residual starch, increased the antioxidant activity, and further stimulating LAB growth during fermentation. This selected starter culture was also employed to produce a functional beverage from maize and a blend of maize and rice added of prebiotics (Freire et al. 2017a). According to these authors, those beverages were acceptable by the consumers that may be associated to the chemical compounds detected, such as lactic and acetic acids and some esters, merged to provide the unique taste and aroma of the product.

Most of the advances in the use of starter culture for ethnic foods and beverages are regarding nonalcoholic beverages and foods or alcoholic rice-based beverages produced in Asia. In Asia, the preparation and the use of fermentation starters as a source of inoculum are important in the manufacturing of alcoholic rice-based beverages. It is recognized by producers that the choice of starter tablets influences the yield and quality of the beverage. Many local producers claim that using a combination of two or three different starters yields beverages of better quality with a stronger sweet alcoholic taste and more attractive flavor than is obtained with a single starter.

Each starter has its own advantages and disadvantages. These dried starters normally include yeasts, fungi, and bacteria and convert starchy materials to fermentable sugars and subsequently to alcohol and organic acids. A variety of starter cultures is available in the markets in most Asian countries, for example, *Saccharomycopsis* for *marcha* and *Mucor* spp., *Rhizopus* spp., *Aspergillus* spp., and *Penicillium* spp. for *nuruk*.

In America, the ethnic fermented foods and beverages are prepared without using inoculum. In general, the microbial diversity found in different fermented beverages may be associated with the source (e.g., substrate and process) as well as the fermentation conditions that provide a particular environment for the growth of the microbial communities. In addition, the contaminant microorganisms may be also associated with the environment, the water, cleanliness of producer's hands, or nonsterile utensils used to produce the food. The microbial communities present in each fermented beverage produce metabolites that confer the particular sensorial characteristics of the beverages. Table 3.1 shows some volatile compounds detected in different traditional fermented foods and beverages. Therefore, more studies about role and technological characterization of microbial strains from American fermented foods and beverages are required in order to standardize and better control these kinds of fermentations and products.

5 Final Remarks

Different substrates, especially rich-starch substrates, such as maize, rice, and cassava are traditionally used to produce ethnic fermented foods and beverages in America. The substrate, processing and production way is variable which confer individual characteristics to the beverages. In general, the productions of traditional foods and beverages are a homemade art, performed by small communities and indigenous tribes. These fermentations are dominated by yeasts (especially *S. cerevisiae*) and bacteria (BAL and *Bacillus* spp.), which probably come from substrate, environment, utensils, or even from the manipulator's hands. Knowledge and divulgation of these beverages are of great importance in the preservation of identity these peoples as well as for a better understanding of the diffusion of culture society in general. The number of bibliographies on indigenous foods and beverages in recent years has increased; however, studies focusing on the improvement of their production techniques are still scarce. Researchers have challenges in future to seek more information on current technologies used for fermentation as well as on developing novel technologies, so as to obtain high-quality products, which will be appreciated by the consumers worldwide.

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4

Traditional Fermented Food Products of Turkey

Gözde Konuray and Zerrin Erginkaya
Cukurova University, Faculty of Agriculture, Department of Food Engineering, Adana, Turkey

1 Introduction

Over the past few years, traditional food products, particularly those registered as geographical indications, have gained great popularity due to their local identity, the transfer of cultural heritage to future generation and good marketing potential (Sofu and Yeşim Ekinci, 2016). Traditional foodstuffs enable the connection between today and history of a culture (Kaban, 2013). Traditional foods are based on basic processing methods for centuries and their shelf life is not long compared to today's processed products (Kocatepe and Tırıl, 2015). Moreover, people recently have switched their attention to consume therapeutic and nutritional foodstuffs containing natural and organic ingredients including herbs and spices because of the growing awareness on the health benefits (Sofu and Yeşim Ekinci, 2016).

Nowadays, fermented foodstuffs are drawing attention of consumers (Karaçıl and Acar Tek, 2013). Fermentation technology is an old and economic method that is used in production and storage of food. Fermented products are being consumed widely throughout the world due to their beneficial effect on health (Kaban, 2013; Levent and Cavuldak, 2017). It is one of the cheapest and easiest methods to increase the shelf life, nutritional value, sensory properties, and quality of food and has been in use since ages (Kabak and Dobson, 2011; Levent and Cavuldak, 2017). The nutrition content of food products naturally increases with fermentation (Kabak and Dobson, 2011). Lactic acid fermentation gives food advantages compared to non-fermented products such as high nutritional value and easy digestibility. As a combining result of reducing pH and potential of oxidation-reduction, producing of antimicrobial products, and competing for nutrition source, the growth of many pathogenic and spoilage microorganisms is inhibited (Hancıoğlu and Karapınar, 1997).

Lately, traditional fermented products are drawing attention with its special characteristics (Sofu and Yeşim Ekinci, 2016). Besides their high nutritional value, they have a beneficial effect on consumers' health (Kabak and Dobson, 2011). Traditionally fermented beverages and food products consist of various microflora that contribute to forming of aroma and texture (Sofu and Yeşim Ekinci, 2016).

Due to being an intersection point between various regions and cultures, the traditional cuisine of Turkey has influenced different kinds of food (Weichselbaum et al., 2009).

2 Fermented Dairy Products

Fermented dairy products can be produced with fermentation by microorganisms (Kabak and Dobson, 2011). Lactic acid bacteria play an important role in the production of traditionally fermented dairy foodstuffs (Kabak and Dobson, 2011). Yeasts are also known to contribute to the texture, aroma, and nutritious value of many traditional dairy products (Kabak and Dobson, 2011).

2.1 Yogurt

One of the utilizing methods of milk is yogurt production (Atasever, 2004). Yogurt is a traditional fermented dairy product that is produced by fermentation of milk (Kabak and Dobson, 2011; Innocente et al., 2016; Tian et al., 2017). The milk of cow, buffalo, goat, and ewe is used for the production (Erkaya and Şengül, 2011). It is being produced in Turkey for many years (Kabak and Dobson, 2011). The fermentation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* occurs in the production (Kabak and Dobson, 2011; Innocente et al., 2016). Curd, which is produced by coagulated milk proteins, forms the yogurt. Yogurt production in Turkey is generally maintained with unstandardized methods (Atasever, 2004). In addition to the proteins, minerals, and vitamins, it contains calcium in high quantity (Gezginc et al., 2015). Due to the increasing consumer interest on dairy products that have beneficial effect on health, yogurt production has improved recently (Erkaya and Şengül, 2011; Innocente et al., 2016).

Yogurt production is generally homemade in Turkey. The traditional method uses the following steps for yogurt production: first, milk is boiled until the milk volume reduces to one-third of its original volume, then it is cooled down to the body temperature, and then a small piece of yogurt which remained from previous production is added, and it is kept at stable temperature for a while and then cooled down (Biberoğlu and Ceylan, 2013). The milk, which is used in the yogurt production, plays an important role in the texture, aroma and nutritional value of yogurt (Erkaya and Şengül, 2011).

Concentrated yogurt is one of the oldest and most important foods of traditional Turkish and Anatolian cuisine (Şimşek et al., 2010). It is concentrated by stuffing into animal skin or cheesecloths. But today it can be produced industrially in hygienic conditions by traditional method or use of various separators (Şimşek et al., 2010).

2.2 Cheese

Cheese has an important role in Turkish cuisine. Many different cheese varieties, which have various taste, shape and texture, can be found (Durlu-Özkaya and Gün, 2014). Turkey plays an important role in the production of cheese producing more than 50 types of cheese (Özmen Toğay et al., 2016; Temiz and Kiliç, 2016; Karabey et al., 2018). Various cheese types can be found in small-scale enterprises in Turkey, which is produced through traditional techniques and spontaneous fermentation without starter culture addition (Sönmez et al., 2018). In addition to principal cheese such as white cheese, kashar cheese, curd cheese, and cottage cheese, local cheese are also produced such as abaza, herby cheese, mihalic, string cheese, sirvatka, hellim, circassian cheese, basket cheese, ezine cheese, cayir cheese, and civil cheese (Özmen Toğay et al., 2016; Temiz and Kiliç, 2016).

White cheese is the most important cheese produced in Turkey (Öner et al., 2006; İspirli et al., 2017). Its annual production represents 60% of the total cheese production (Salum et al., 2018). White cheese is a soft or semi-soft cheese (Öner et al., 2006). In addition to being industrially manufactured, it can also be produced in small scale (İspirli et al., 2017). Traditionally, goat and sheep milk is used in the production, but in case of inadequate supply of these milk, cow milk or mixture of these milk is used in the production (Hayaloglu et al., 2002; Salum et al., 2018). Although it has a soft texture, it gains a semi-soft or semi-hard texture after 3 months of ripening in brine (Öner et al., 2006).

Kashar cheese is the other most important of the traditional Turkish cheese (Yuvaşen et al., 2018). It is produced with raw sheep milk, raw cow milk or the mix of these milk (Aydemir et al., 2015). Kashar cheese is produced traditionally in Kars city, which is located in the North East of Turkey (Aydemir et al., 2015; Yuvaşen et al., 2018). It has a cylindrical shape with a diameter of 30 cm and a height of 20 cm and a weight of 12 kg (Aydemir et al., 2015). The kashar cheese is produced without the addition of a starter culture in the small-scale manufacturing sector. In the traditional production of kashar cheese, raw milk is filtered through cheesecloth and transferred into the cans. Subsequently, milk is heated up to 30–33°C and rennet is added. When the milk is coagulated, the curd is transferred into the cheesecloth. After the filtration, the curd is cut and grated. The grated curd is cooked in water at 70–80°C for 2–3 min in a metal basket. The cooked curd is kneaded like a dough, transferred into a round metal container and stored for 12–24 h. Salt is sprinkled on the cheese and placed on wooden shelves in the ripening room. Cheese is periodically inverted and stacked. After the first ripening period, which takes 3–4 weeks at room temperature, five or six cheese is stacked into a bag and stored in a cold room (3–4°C) from 1 month up to 2 years for second ripening stage (Aydemir et al., 2015). No starter culture is added during these two maturation periods (Yuvaşen et al., 2018).

The tulum cheese, which is traditionally produced from raw milk, is a popular Turkish cheese (Bayar and Özrenk, 2011). Except for the Thrace region, it is produced in every region of Turkey (Bayar and Özrenk, 2011; Karabey et al., 2018). Because the applied techniques are different in each region, the quality of the cheese is not standard (Bayar and Özrenk, 2011). Cow, sheep, or goat milk is used in the production process. Its color is white or cream and it has a crumbly, semi-hard or hard texture, and has a piquant and greasy taste. This cheese is called tulum because of its ripening period that occurs in a goat or sheep skin. Since the skins are water and air permeable, it gives hard texture and characteristic taste and flavor (Karabey et al., 2018). In the past, goat skin is commonly used in the ripening period, but due to the difficulty in obtaining the skin, nowadays alternative containers are being used, which are made from wood, plastic, or soil (Bayar and Özrenk, 2011; Karabey et al., 2018).

Curd cheese has various names according to its production region such as basket curd, herby curd, sirvatka, şor and Tire mud cheese (Kavas and Kavas, 2016). Curd cheese is made from whey and it is a soft cheese (Temiz et al., 2009). At first, whey is heated up to 50–55°C. After separating the oil, it is heated up to 80°C and 2% salt is added. The temperature is increased to 90–95°C. The coagulants of curd cheese are drained with a cheesecloth for 12 h at 25°C (Irkın, 2011). Generally, it is consumed as unripened (Temiz et al., 2009). The natural microflora is not resistant to the production temperature. Thus, the contamination, when occurs after the production, is a safety risk factor for the cheese (Kavas and Kavas, 2016). Curd cheese is generally sold and transferred as unpacked. As a result of this, it is exposed to non-sterilized conditions in the market. Therefore, the shelf life of curd cheese is less than 7 days (Temiz et al., 2009).

Cottage cheese is a white colored formless cheese and has a crumbly texture and characteristic flavor. Although it is commonly produced in small-scale enterprises, traditionally, it is one of the five main cheese types produced in Turkey (Celem et al., 2018). Its composition changes according to raw material, production technique and consuming type (fresh or ripened) (Kavas, 2017; Celem et al., 2018). Cottage cheese generally is produced by boiling the yogurt and filtering it using cheesecloths (Özbek and Güzeler, 2017; Celem et al., 2018). Milk, buttermilk or whey mixed with yogurt is used as raw material. Butter, milk, salt and various herbs could be added in the different stages of production, for the purpose of enrichment of the aroma (Celem et al., 2018). Cottage cheese contains high calcium and water content and less oil content, and it also contains casein and serum protein together, due to the heat treatment applied (Kavas, 2017). It contains significant amounts of casein and whey proteins. It is an important source of animal protein due to its low calorie and cheap cost (Celem et al., 2018).

2.3 Ayran

Ayran is one of the important fermented beverages that is being producing for many years in Middle Asia and Anatolia and has a special place in the diet of the Turks (Patır et al., 2006; Altay et al., 2013). It can be produced as homemade or industrially (Altay et al., 2013). It could have short or long shelf life depending on the production method (Şeker and Patır, 2011). For homemade produced ayrans, it has a short shelf life (Altay et al., 2013). It can be produced with mixed yogurt, salt and water in homemade production (Kabak and Dobson, 2011; Akkaya et al., 2015). Its industrial production is performed through the fermentation of milk by *Streptococcus* *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Altay et al., 2013). The fermentation stops when the pH value of product reaches to 4.4–4.6. Water and salt are added to the product for dilution (Kabak and Dobson, 2011). After the fermentation step is stopped with the heat treatment, it can be stored at room temperature for 30 days or at 4°C for 60 days (Şeker and Patır, 2011).

It has a high nutritional value with calcium and vitamin (Altay et al., 2013). Generally, it is composed of water (94%), total solids (5%), fat (1.2%), protein (1.7%), salt (0.7%) and ash (1%) (Kabak and Dobson, 2011).

Due to its refreshment characteristic, it is commonly consumed in Turkey especially in the summer (Akkaya et al., 2015). According to the Turkish Statistical Institute, ayran is the fourth most consumed dairy product (the most consumed products are milk, yogurt, and cheese in the hierarchy) in Turkey in 2015 (Türkmen et al., 2017). Its viscosity and mouthfeel play an important role in the consumers' preference and demand (Engez et al., 2006).

2.4 Kefir

Kefir is obtained with the fermentation of milk by lactic acid bacteria, acetic acid bacteria and yeast which are found in 'kefir grains' (Kabak and Dobson, 2011; Çetinkaya and Elal Mus, 2012). Ethyl alcohol and lactic acid fermentations occur in the production (Karaçıl and Acar Tek, 2013). It has acidic characteristic and a flavor barely recognizable as alcohol and gives freshness (Öner et al., 2010; Karaçıl and Acar Tek, 2013). Fermentation starts with addition of the kefir grains which could be divided as traditional grains or commercial grains (freeze-dried starter cultures) (Yüksekdağ and Beyatlı, 2003; Çetinkaya and Elal Mus, 2012). Kefir grains are white-cream in color, and their diameters vary between 1 and 6 mm. They look like cauliflower and their size is similar to pea or hazelnut. Kefir grains do not dissolve in water. They become inflated and their color turns white when they are transferred into the milk (Yüksekdağ and Beyatlı, 2003; Öner et al., 2010). Kefir grains consist of casein and commensal microorganisms which generate gelatinous colonies. The most important feature of kefir grains is that after the fermentation, they can be recovered by filtration and reused (Yüksekdağ and Beyatlı, 2003).

Cow, sheep, goat and camel milk could be used in the production. In the traditional kefir production, homogenized milk is pasteurized and heated up to 90°C for 5–10 min. After the heating process, milk is cooled down to 20–25°C. The kefir grains are transferred to the cooled milk. The fermentation occurs for 18–24 h at 20–25°C. The kefir grains are removed through a cloth after the fermentation is stopped. Kefir has an acidic pH and can be stored at 4°C (Kabak and Dobson, 2011).

Kefir contains probiotic microorganisms (*Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Saccharomyces, Kluyveromyces* and *Torula*) and their products (Çetinkaya and Elal Mus, 2012; Tarakçı et al., 2015). The aromatic molecules give kefir its own sensory characteristic which is produced as a result of fermentation of milk by lactic acid bacteria and yeasts (Karaçıl and Acar Tek, 2013). Milk composition, milk amount, starter culture type, heat treatment, homogenization, incubation temperature and time, cooling process and storage conditions play an important role in the physicochemical characteristics of kefir (Taş et al., 2011; Ergin et al., 2017). Kefir has a refreshing characteristic and can be consumed with fruits, sauce, soup and cakes (Goncu and Alpkent, 2005; Karaçıl and Acar Tek, 2013).

2.5 Koumiss

Koumiss is a traditionally fermented beverage produced from mare milk and has a milky-grey color (Kabak and Dobson, 2011; Karaçıl and Acar Tek, 2013). Koumiss is recognized as the beverage of God by Turks in ancient times (Karaçıl and Acar Tek, 2013). Since fermentation maintains when koumiss is prepared for consumption, its nutritional value is not stable. In traditional production method, mare milk is immediately fermented after milking. A small part of previous production can be used as starter of new fermentation (Karaçıl and Acar Tek, 2013).

2.6 Dried Yogurt (Kurut)

Kurut is a fermenteddairy that made by desiccation of yogurt and ayran. It is produced especially in the Eastern Anatolia region of Turkey and consumed with soup, meat pasty or some traditional foods (Kabak and Dobson, 2011; Karaçıl and Acar Tek, 2013). Kurut production is performed during the period when milk is in abundance (Karaçıl and Acar Tek, 2013). Yogurt or ayran is dried under the sun in the traditional production methods (Kabak and Dobson, 2011). Kurut has a sourish taste. If it mixed with water, it gains a viscose texture such as yogurt. Kurut is produced in order to meet the demand for yogurtin in the absence of milk in winter. It can be stored for 1 year in humid-free and cold environment (Kabak and Dobson, 2011; Karaçıl and Acar Tek, 2013). Kurut contains high amounts of protein and minerals (potassium, calcium, phosphorus) (Kabak and Dobson, 2011).

3 Fermented Meat Products

Fermentation is a rather an old method to preserve meat and produce novel foodstuffs (Geçgel et al., 2016). Each country has fermented meat foodstuff which has its own characteristic taste and aroma. Today, the primary aim in the production of fermented meat product is to produce foodstuffs with the taste desired by consumers (Karaçıl and Acar Tek, 2013).

3.1 Turkish Sausage (Sucuk)

It is a traditional foodstuff that is produced in small scale for centuries in Anatolia (Kabak and Dobson, 2011). In Turkey, traditional Turkish sausage (sucuk) is the first that comes to mind among fermented meat products. It is the most preferred one although the recipe of sausage shows differences between regions (Kılıç Altun et al., 2015; Geçgel et al., 2016).

Ground meat (from sheep, beef of water buffalo) salt, fat (from sheep tail or beef), garlic, nitrate, nitrite, spices, sugar and other supplements are used in the production. All the ingredients are mixed and filled into the small intestine of a cattle. Then, it left to fermentation in a dry air environment at 18–22°C for 21–25 days (Demirezen and Uruç, 2006; Ercoşkun and Özkal, 2011; Kaban, 2013). After the drying step, its fat component increases from 10–20% to 30–40% (Kayaardı and Gök, 2003). Its fat content and pH should be lower than 40% and 5.4, respectively (Kılıç Altun et al., 2015).

Due to the effect of microorganisms on aroma formation, pH, stability and safety of the product, they play a significant role in the sucuk quality (Kilic, 2009). In food industry, starter culture used in sucuk production is becoming widespread (Kaban, 2013). In small-sized enterprises, meats, which contain natural microflora, are fermented without use of starter culture (Başyiğit et al., 2007). Due to its characteristics, consumers generally demand the traditionally fermented sucuk (Kaban, 2013). These characteristics depend on the raw meat quality, natural microflora and technology used in production (Başyiğit et al., 2007).

4 Fermented Vegetable Products

Today, although cooling, freezing and canning applications are developed in many countries, fermentation is the most practical and common method to protect the products in undeveloped and developing countries. Many fermented vegetable products have acidic characteristics due to the production performed by the lactic acid fermentation (Karaçıl and Acar Tek, 2013).

4.1 Shalgam

Shalgam is a quite popular fermented Turkish beverage in the southern parts of Turkey. It is a cloudy, red colored beverage. Shalgam is generally produced as homemade but its industrial production is also maintained (Erten et al., 2008). Different techniques used in production lead to differences in product stability and quality (Altay et al., 2013). There are two fermentation steps in the traditional shalgam production. The first fermentation step is defined as sourdough fermentation and it is carried out with the dough which is obtained with mixture of water, salt, bulgur flour and sourdough. It is left to fermentation at 25°C for 3–5 days. Then the dough is extracted with water four times and the extraction is transferred to the fermentation tank where the second fermentation (carrot fermentation) will occur. Salt, water and carrots were added into the fermentation tank and left to fermentation at 25°C for 3–10 days. After the fermentation, the product is filtrated and transferred into bottles. Shalgam is stored at 4°C (Üçok and Tosun, 2012; Altay et al., 2013).

4.2 Pickle

Pickle is a product that increases its importance due to consumer demand to natural and additive-free products (Tokatlı et al., 2012). Cucumber takes the first place among vegetables that are used to produce pickle in Turkey (Uylaşer and Erdem, 2004). Furthermore, cabbage, tomato, carrot, bean, and pepper are commonly used for pickle production (Karagöz and Güllü, 2017; Tokatlı et al., 2017). Also in different regions, other various vegetables and fruits are used for pickle production depending on the demand of locals, such as apple, olive, plum, unripe melon, apricot, okra, cauliflower, pear, grape, broccoli and kapari plant (Karagöz and Güllü, 2017). Pickle is predominately produced as homemade or in small-scale enterprises in Turkey (Uylaşer and Erdem, 2004). In traditional pickle production, vegetables are washed, prepared for fermentation (separation of stems and leaves, size reduction) and transferred into a suitable container. Brine, which is previously prepared with salt, garlic, vinegar and water, is added to the container. Then it is left for fermentation at room temperature (Çetin, 2011).

4.3 Hardaliye

Hardaliye is a traditional fermented beverage. It is prepared with the juice of grape, crushed black mustard seed and cherry leaf (Kabak and Dobson, 2011; Altay et al., 2013). It is produced in the Thrace region of Turkey (Soyuçok and Başyiğit Kılıç, 2017). Red (black) fragrant grapes are used in the production when they reach sufficient maturity. Hardaliye is generally produced in October and November, when the vintage season starts. The grapes are separated from cluster and washed, and then transferred into a barrel. Crushed black mustard seed, cherry leaf and potassium benzoate are added to the crushed grapes. The mouth of the barrel is left open. After the fermentation starts, it must be taken from the tap under the barrel and poured again on the barrel in every two days. It is stored in the room temperature for 7–10 days. At the end of the fermentation, the product is filtered and filled into the bottles. The shelf life of hardaliye is approximately 3 months (Coşkun and Arıcı, 2011).

5 Fermented Cereal Products

Cereal products are the source of vegetable protein, carbohydrate, mineral and pulp in our diet. Fermentation is the easiest and economical way to increase the nutritional value of cereals (Karaçıl and Acar Tek, 2013).

5.1 Tarhana

Tarhana is a fermented traditional foodstuff that is prepared for the winter in Turkey (Kocatepe and Tırıl, 2015; Erinç and Çifçi, 2018). It has a yeasty, sour and acidic taste (Herken and Çon, 2014). It is being consumed gladly by Turks since they live in Middle Asia. It is associated with one of the storage methods of yogurt (Ünver Alçay et al., 2015). Although there are different production methods, it is produced generally with yogurt, various wheat products (such as flour and semolina), *Saccharomyces cerevisiae*, vegetables and spices. The fermentation occurs for 1–7 days. It is dried in the sunlight after the fermentation and finally broken into pieces (Herken and Çon, 2014; Erinç and Çifçi, 2018). Moisture and pH decrease at the end of the fermentation. Thus, there is an unfavorable environment for the growth of pathogens. Tarhana can be stored up to 1–2 years (Karaçıl and Acar Tek, 2013). It could be consumed as a snack, but it is mostly consumed as soup (Karaçıl and Acar Tek, 2013; Erinç and Çifçi, 2018).

5.2 Boza

Boza is a traditionally fermented product which has a viscous structure, characteristic sweet-sour taste, alcohol and acidic smell and a light-yellow color. It is produced with cereals such as rice, wheat, corn, oat, and rye. After the cereals are cooked in water, sugar is added and lactic acid fermentation starts by yeasts (Levent and Cavuldak, 2017; Soyuçok and Başyiğit Kılıç, 2017). A starter culture, which consists of yeasts and *Lactobacillus* spp., is usually used in the boza production. Boza, yogurt or sourdough can be used as the source of starter culture (Altay et al., 2013). Lactic acid and CO_2, the products of fermentation, give refreshing characteristic and taste to the boza (Levent and Cavuldak, 2017).

The difference in cereal type and ratio, and the fermentation conditions can lead to production of different types of boza composition. Although beer and boza are different products in the point of product characteristics and production methods, boza is recognized as the oldest beer. Boza differs from the beer with the low ethyl alcohol composition (< 2%) and lactic acid fermentation in addition to alcohol fermentation (Levent and Cavuldak, 2017).

Boza has been produced by the Middle Asia Turks since ancient times. After Turks migrated to the various regions from the Middle Asia, they introduced boza to the local people and they contributed to its spreading to the different regions. Boza, which is a traditional Turkish beverage, is known by the Balkans, Krym, Caucasia, Middle Asia and Egypt (Levent and Cavuldak, 2017).

5.3 Sour Dough Bread

Bread is an indispensable foodstuff due to its nutritious, satisfying properties and to be provided cheaper and easier compared to other foodstuffs. Sourdough is used in the traditional bread production. After the lactic acid fermentation of wheat, rye or other cereal flours mixed with water, which is performed for 1–2 days, sourdough is obtained. On the basis of the sourdough method, the dough, which in lactic, acetic and citric acid fermentations occur by indigenous and contaminated from air and materials, is used for next production. Sourdough microflora generally consists of yeast (*Saccharomyces cerevisiae*) and a complex mix of heterofermentative and homofermentative lactic acid bacteria. Volume, texture, aroma and shelf life of bread are developed by the use of sourdough. In Turkey, sourdough method is commonly used in some regions due to lack of commercial yeast (Bircan et al., 2017).

5.4 Chickpea Bread

Among the cereal-based fermented products specific to Turkey, there is also the widely known chickpea bread (Yücel Şengün, 2011). It is one of our traditional bread types that has been known since ancient times and produced in Turkey especially in Aegean and Thrace regions and partially in Central Anatolia and Mediterranean regions. The chickpea yeast is obtained by the fermentation of chickpea, salt and boiled water (boiled then cooled down) at 37–40°C for 16–18 h and filtration. The bread, which is produced by the mixture of filtrate, wheat flour and salt, is defined as chickpea bread or sweet yeast bread. Chickpea yeast is quite sensitive and it can be prepared by only crushed chickpea, flour, salt and water (Erginkaya et al., 2016; Sayaslan and Şahin, 2018).

Chickpea yeast contributes to bread dough and makes the dough softer and easier to process. It effects the cooking process positively and the color of inner and outer crust and gives its characteristic taste and aroma (Çebi, 2014).

6 Conclusions

Fermentation is an oldest and economic method to preserve foodstuff and improve its sensory characteristics (Arici & Coskun, 2001; Kabak and Dobson, 2011). Various traditional fermented products have an important place in the nutrition of the majority of population in many countries (Karaçıl and Acar Tek, 2013). Due to its geographical location, Turkish cuisine is influenced by Europe, Ottoman, Arab, Greek and Iranian culture and has a wide range of products (Kilic, 2009). There are many traditional fermented foods and beverages available produced in small scale in Anatolia (Kabak and Dobson, 2011). In recent years, besides traditional fermented products, there has been an increased interest in many fermented foods and beverages produced using a wide variety of raw materials, production techniques and microorganisms. Expansion of microbiological and biochemical researches related to these products, as well as their relations with health, needs to be emphasized (Karaçıl and Acar Tek, 2013).

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Processed Lichens Could be a Potential Functional Food with Special Reference to Traditional Dishes

Mathews Lurth Raj D
Department of Biotechnology, Kamaraj College of Engineering and Technology, Madurai, India

Shakena Thajuddin
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

Ganesh Moorthy I
Department of Biotechnology, Kamaraj College of Engineering and Technology, Madurai, India

Dhanasekaran D
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

Shyam Kumar R
Department of Biotechnology, Kamaraj College of Engineering and Technology, Madurai, India

Thajuddin N
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

Introduction

Lichens are a unique and diversified organisms, living in a symbiotic relationship with a fungal partner (mycobiont) and one or more photosynthetic partner (Algae or Cyanobacteria) (Upreti et al. 2005; Kumar et al. 2011). The total lichen species and lichenized fungi diversity are approximate estimation of 20,000 species worldwide till record. Lichen's structural organization is dominated by mycobionts with 90% responsible for providing the color, structure and texture to the lichen. It also believed that during this symbiosis, fungi provides shelter to the algae and in return algae plays as a food production unit to serve their nutritional requirements. Lichens can survive in any extreme environment from searing hot desert to freezing cold environment nor like algae and fungus which fail to sustain their growth in extreme environmental conditions. Lichen resistance and growth against extreme calamities may be obtained due to their mutualistic relationship. The fungi involved in lichenization mostly Belonged to Ascomycota family and very few from Basidiomycota and other mitosporic fungi (Lutzoni and Miadlikowska 2009). Alike fungi, the mycobiont partner of lichen also consists of a branching filamentous structure called hyphae. The algal cells are sandwiched between the cortex and medulla layer of lichenized fungi surrounded with hyphal filaments. Whereas the cyanolichen (Photobiont is cyanobacteria), the algae and fungal hyphae are uniformly distributed. Lichen forming cyanobacteria performs a dual function by involving in both photosynthesis and nitrogen fixation (Kumar et al. 2010). However, the relative significance of these functions varies in different cyanolichens. It is also believed that cyanolichen fix atmospheric nitrogen and contributes a significant amount to the ecosystem, especially the corticolous cyanolichen forms. Throughout history, people have used lichen for making food, food-related additives, clothing dye, medicines, and tanning agent. It also true that various species of lichen are fit for human consumption, especially during famine and winter season as indigenous people of North Europe depend on lichens for their survival. From ancient times, lichens are traditionally used in Indian folk medicine. Several metabolites isolated from various lichen are proven to have bioactivity such as usnic acid (anticancer) (Kosanić and Marijana 2013), lecanoric acid, atranorin (anticancer) (Zhou et al. 2017), salazinic acid (antimicrobial) (Candan et al. 2007), vulpinic acid (neuroprotective) (Fernández-Moriano et al. 2015), Evernic acid (antioxidant) (Fernández-Moriano et al. 2017), and imbricaric acid (anti-inflammatory) (Oettl et al. 2013). Similarly, few lichen species are also used as biological indicators for measuring air purity and ecological quality to check the environmental health (Dwivedi et al. 2007). However, many of the lichen have been adversely affected by habitat loss, and other human-induced environmental changes have led to reduction in lichen biodiversity.

Lichen Classification and Its Identification

Lichens are classified based on the mycobiont involved in lichenization and its components. The large groups of fungi involving in lichenization are ascomycetes and very few basidiomycetes. Identification of lichen is based on the thallus morphology, structural arrangement, biochemical test, spore formation, and its arrangement. The monographs and lichen identification key were introduced by pioneer lichenologists Awasthi (1965, 1975, 1988), Hale (1969), Hawksworth (1974), Upreti (1987), and Orange (2001).

The characteristic feature observed from the mycobiont components plays a significant role in categorizing the order, family, and genus of lichens. The key features include asocmatal structure, ontogeny of Ascomycota, Ascospore types, spore septation, and its arrangement which help in identifying the species. In addition, chemical constituents and their presence or absence in lichen thallus are also a stable method for taxonomic criteria for species identification (also known as spot test) (Orange 2001). There are different chemical constituents specific to the species living in a particular geographical origin; such lichens are categorized as subspecies.

Spot Test

Although lichen can be readily identified using various macroscopic and microscopic observations, still the chemical test plays a vital role in species identification. A few chemical reagents like K (potassium hydroxide), C (Bleach), P (para-phenylenediamine) and KC (potassium hydroxide treatment followed by bleach) were used and these tests were performed by wetting the thallus and color changes were observed. Such spot test and chromatographic separation of crude lichen extract are a precise method for species identification in few lichens (Nayaka 2014).

Lichen Classification Based on Their Thallus Morphology

Lichen morphology and their growth forms were used to broadly categorize lichens. The lichen thallus is the vegetative portion with a diverse range of structures and is classified as Crustose, Foliose, Squamulose, Fruitocse, and Leprose. The thalli with diversified forms carry multiple tufts, leaf-like lobules arrangement, and powdery granules such as soredia and isidia on its surface.

Crustose

Crustose form lichens have a crust-like appearance tightly adhered to their substrate by their lower surface. They do not possess lower cortex in thalli. A crustose form has two-dimensional growths, making a total of 75% in the population of all lichens on earth. A few examples of crustose lichen are *Pyrenula, Caloplaca* and *Graphis* sp.

Squamulose

These are the lichen with small flat thallus, usually massed and overlapping scales. Squamulose are in-between forms of foliose and crustose. The lichen thallus is completely adhered to from the lower surface, but free at the tips. Alike crustose form, squamules also do not have a lower cortex, but sometimes few genus produce upright branched structure called podetia. A few examples of squamulose lichen are *Cladonia, Ophioparma*, and *Squamarina.*

Foliose

The lichen thallus generally has flat leaf-like lobes with differentiated layers of tissue. The lower surface is lifted above, free from attaching and only the center portion is adhered to. The lower cortex in foliose is often colored along with small root-like structure called rhizines. The thallus of foliose lichen is obviously three dimensional in growth. Foliose lichen are highly sensitive to the pollutants such as sulphur dioxide. Sulphur dioxide reacts with chlorophyll present in lichen photobiont and produces phaeophytin and magnesium ions, which leads to an abrupt decrease in chlorophyll-based respiration that ultimately kills the lichen. A few examples of foliose lichen are *Xanthoparmelia, Evernia, Parmelia*, and *Dirnaria.*

Fruiticose

Lichens having leafless branches maybe upright and shrubby with pendulous strands. Obviously, three-dimensional thallus growths with ribbon-like flattened or twig-like tufts are usually observed. Usnea is a fruiticose lichen which has the hanging and tassel like thalli. Similarly, Cladonia lichen with stiffy, erected branches resembles tiny buds. Few well-known examples of fruticose lichen are *Usnea, Cladonia*, and *Ramalina.*

Lichen Reproduction

Lichen reproduction is carried out either by vegetative or sexual form of reproduction. The fragment of lichen carrying both photobiont and mycobiont may get separated and develop into new lichen; this may occur when the thallus is abruptly broken or fallen off. Such fragments that often fall and involve in asexual reproduction in lichen are commonly isidia and soredia. Isidia are stalked and may be branched or unbranched outgrowth formed in the thallus. Similarly, soredia are round powdery vegetative body found in lichen on the surface of thallus which also responsible for asexual reproduction (Bowler and Rundel 1975). The lichen undergoing sexual reproduction is mostly by the tiny spores produced within an ascus in a specialized structure called ascomata. Apotheicium is a common type of ascomata with cup-shaped openings carrying ascospores arranged sequentially. The sexual reproduction through the spores only can regenerate the fungal partner, whereas the germinated spore should find its own suitable photobiont partner to form new lichen.

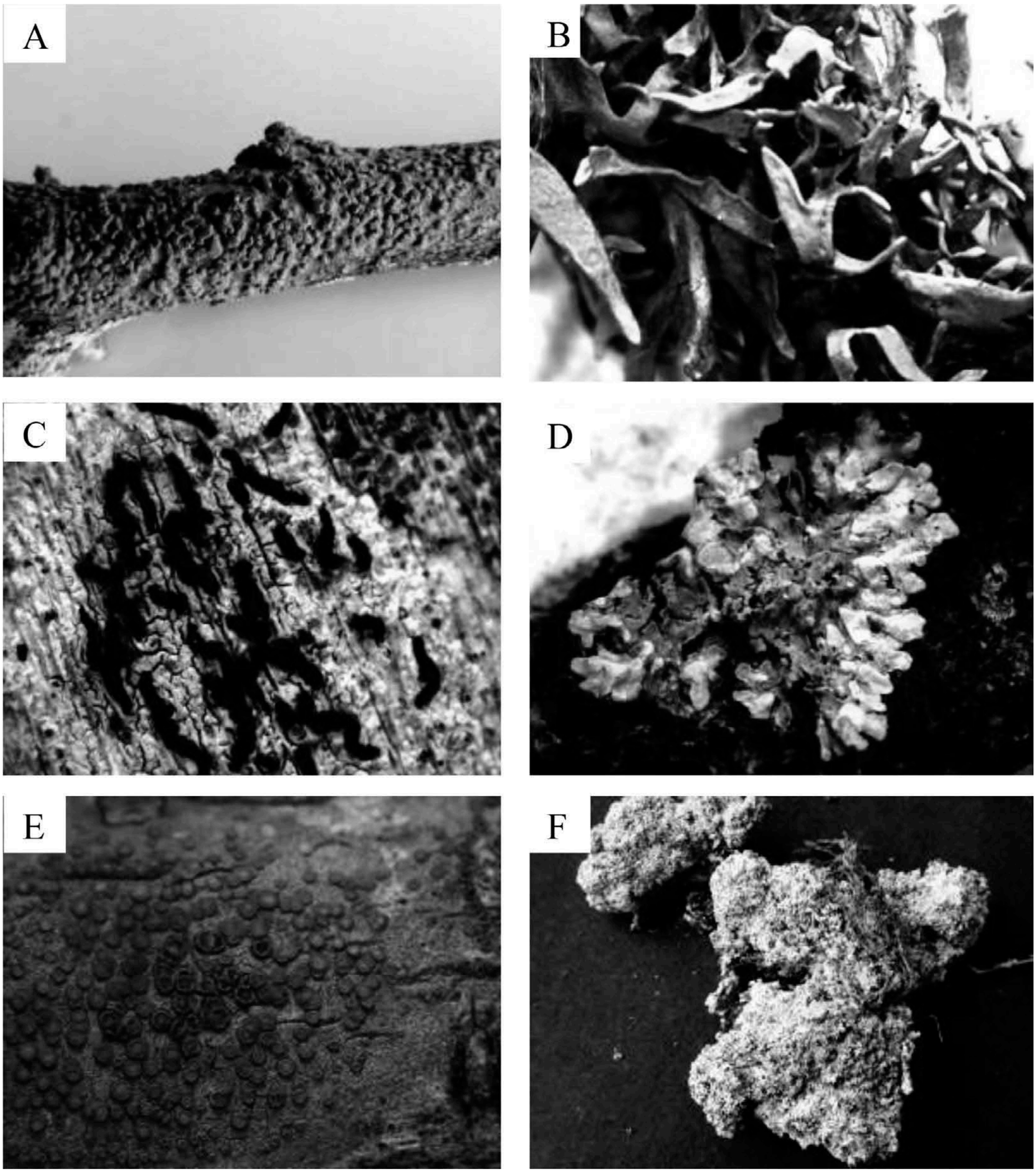

FIGURE 5.1 Sterozoom images of lichens collected from coastal districts of Tamil Nadu.

A) *Trypthelium* sp. (Crustose), B) *Roccella* sp. (Fruiticose), C) *Lecanographa* sp. (Crustose), D) *Pyxine* sp. (Foliose), E) *Lecanora* sp. (Crustose), F) *Cladonia* sp. (Squamulose)

Lichen Habitat

Lichen is found in all diversified habitats ranging from hot temperate desert to cold Antarctica, tropical forest, mountains, and seashores. In addition, they grow on many different substrates. Few lichens are substrate specific, and therefore based on the lichen's growth on the substrate, they are classified as: a) Corticolous (Lichens on bark), b) Lignicolous (on any organic material), c) Foliicolous groups (on leaves), d) Saxicolous (on rock, concrete), e)

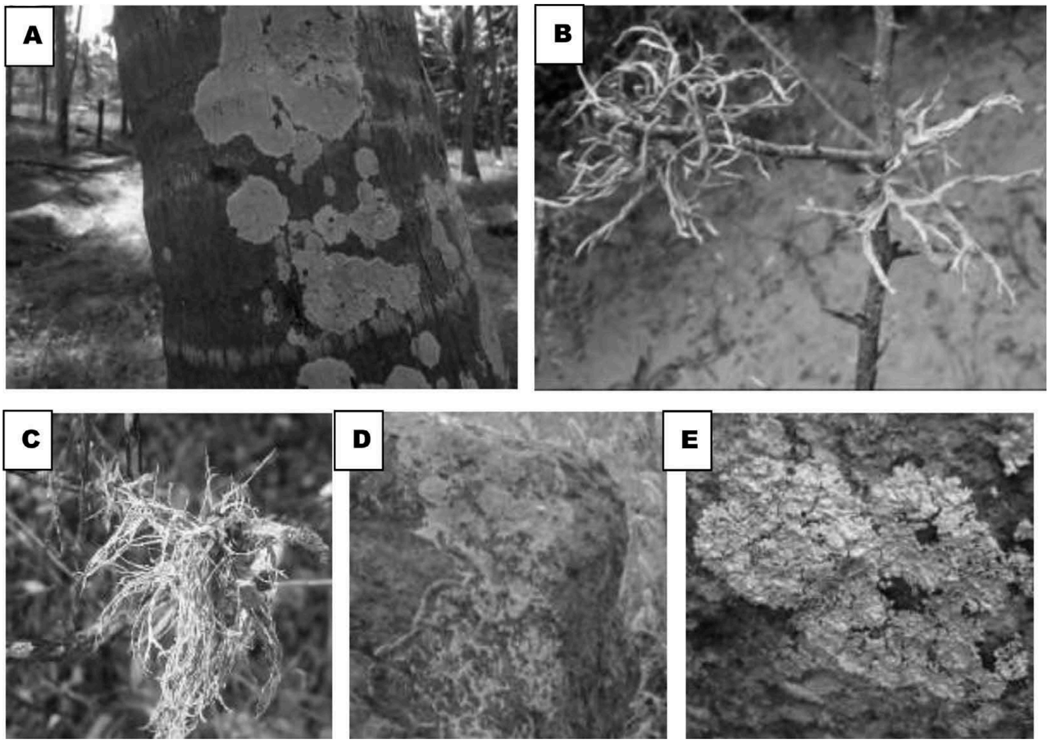

FIGURE 5.2 Lichen and their substrates or habitats. A) Lichen *Dirnaria* sp. in coccunut tree (Corticolous). B) *Roccella* sp. in *Prosopis juliflora* (Corticolous), C) *Ramalina* sp. in *Avicenniae* sp. (Corticolous) D) & E) *Dirnaria* sp. in rocks (Saxicolous).

Terricolous (on soil), and f) Muscicolous (on moss). Similarly, lichen also remains a habitat for several small creatures and performs various useful functions for the nature. Lichens are taken as food by various animals like reindeer and caribou, especially in winter months. The lichen materials are also used by various birds and small creatures to build their own nest or home. A saxicolous form of lichen and their chemical constituents released are reported to have slowed down the breakdown of rock and soil formation.

Lichens as Food Source for Human

Food culture in eastern Himalaya has a dish named Yangben or Limbu made from lichen parmelia boiled with wood ash and consumed as dry curry, and is a well-known staple food of Nepalis (Limboo and Rai) of eastern-Nepal (Tamang 2001). Ethnic food culture and indigenous knowledge of food production with nutritive qualities have been reduced. Similarly, lichen *Bryoria fremontii* and Parmeliaceae harvested from coniferous tree branches of higher altitude were cooked with camas bulbs (onion type) by the people of western North America, which was adopted as an emergency food by explorers and settlers. Likewise *Actinogyra* sp. and *Umbilicaria* sp., including some formerly classed as *Gyrophora* sp. were a fastidious emergency food used by communities like Cree, Chipewyan, and other Indigenous Indians especially during the extreme shortage of food and famine.

All lichens are not suitable for eating and a few are believed to be mildly toxic to mankind. Especially, few lichens are indigestible when eaten raw due to the excess lignin present in it. However, several communities have learned to process the lichen and making suitable to eat and even make it

TABLE 5.1

Few edible lichens and their country of origin

Sl.No	Edible lichen	Community/Country	Dish	Reference
1	*Parmelia*	Limboo and Rai community of Eastern-Nepal	Dry curry	Tamang 2001
2	*Bryoria fremontii* and *Parmeliaceae*	Western North America	Dry curry with onions	Turner and Aderkas 2012
3	*Actinogyra* and *Umbilicaria* sp.	Western Canada	Vegetables	Kuhnlein and Turner 1991
4	*Parmotrema*	Tamil, Telugu peoples, south Indian	Spices	Upreti et al. 2005
5	*Cladonia rangiferina*	North America	Along with egg and fish curry	Coxson 2015
6	*Umbilicaria esculenta*	Asian and Korean Buddhism	As salad along with berries	Kim et al. 2006
7	*Bryoria* sp.	Tribes of british columbia	Staple food	Hale et al. 1988.
8	*Umbilicaria* (rock tripes)	Japan	Soup	Hale et al. 1988.
9	*Ramalina himalayensis*	North East India/Bugan and Monpa tribals	Used as vegetable	Saha et al. 2014
10	*Alectoria* sp. or black lichen and Barbequed Black lichen)	Black lichen (Alectoria species/Warm spring Indians)	Used as dessert	Hilty et al. 1980

more delicious. Even in south India, the lichen *Parmotrema* commonly known as Kalpasi in Tamil has been regularly used as a spice while preparing dishes like briyani and other non-vegetarian dishes to make them more delicious (Upreti et al. 2005). However, people still lack knowledge of choosing the lichen and the exact processing method. Whereas several other local communities of North America are still using lichen as their staple food. Reindeer moss, or *Cladonia rangiferina*, is a fruticose lichen consumed as a staple food of reindeer and caribou (few called it 'moss' but it's actually lichen). Besides, certain Arctic inhabitants process the partially digested lichen from caribou stomachs and mix with raw fish eggs and they call it 'concoction' which means 'stomach ice cream'. Similarly, *Umbilicaria esculenta* is black foliose form of lichen which commonly grows on rocks. This particular lichen is commonly used in Asian cuisine after being fried. Indigenous groups of European country process and boil specific species of lichens and mix them with berries, fish, or wild onions before eating them. There is a rich diversity of lichen with high nutritive values and several others are not identified and only few are tested for edibility.

Lichen as Nutritive Source

Lichens are rich sources for nutritive values. A study by Lal and Rao (1956) found that calcium and iron levels in lichens are higher than other common cereals and green leafy vegetables. Similarly, another study documents that the calcium to phosphorus ratio was found to be higher in various lichens that serve as a good source of calcium. Lichen *Peltigera canina* is reported to have a relatively higher quantity of protein and amino acid production. Lichen has also absorbed chemicals from the environment and helps in reducing the air pollutants. Few species like *Cetraria islandica* and *Cladonia* sp. are found to possess high levels of cadmium, lead, and mercury deposition in the thallus. In addition, Perez-Llano (1944) reported that lichen *Parmelia saxatilis* and *Xanthoria parietina* were found to absorb higher quantity of beryllium from the environment which is particularly poisonous to the animals. Few reports also confirmed the accumulation of natural radionuclides Po-210 and Pb-210, both accumulated in lichen thallus (Airaksinen et al. 1986).

Lichen as Source of Ethnic Medicine

Since several decades, Indians have been traditionally using lichen in their folk medicine. India has a great diversity of lichens and the Indian subcontinent harbors a rich lichen flora of about 2,450 species (Awasthi 2000). Lichen mega diversity areas are Himachal Pradesh and Uttaranchal hills which are prime source for lichen collection. In India, Upreti (2001) documented the ethnic use of lichens in various cultural events in which he reported the Shipal in Atharveda (1500 B.C.) is the first record of the use of lichen as medicine. The well-known lichen used as medicine since ancient times are *Lobaria pulmonaria* and *Cetraria* sp. that are used for treating respiratory disease, *Usnea barbata* used for strengthening the hair and uterine ailments, *Xanthoria parietina* are used for jaundice, *Cladonia* sp. for cold and cough, *Roccella* sp. for treating heart related disease, *Parmelia* sp. for epilepsy, *Peltigera canina* for hydrophobia and several species of *Evernia, Cladonia*, and *Roccella* lichens are used to control fever.

Traditional Delicious Recipe Made with Lichens

(i) **Water gruel**—The moss is steeped in water and cut up using a special curved knife; hot water is poured over the moss; flour or rice is added. The gruel is then boiled and served with milk.)

(ii) **Moss milk**—Whole pieces of lichen are boiled in milk and the mixture eaten as a soup. Iceland moss is used to eke out the flour in bread and in the making of the Icelandic blood pudding, especially in times of great hardship

(iii) **Iceland moss tea** (The lichen is boiled in water, then strained and the stock is used for drinking and was considered very wholesome.) This beverage was also used for medicinal purposes, especially with regard to respiratory illnesses such as colds, pneumonia, asthma, and tuberculosis (Svanberg and Egisson 2012). Some think it best to boil the lichens for only 5–15 minutes, as it is believed to become bitter if boiled for a whole hour; however, after 2–3 hours it takes on a sweet taste.

(iv) **Lichen spices** with *Parmotrema* sp. is an essential ingredient in a mixture of spices and cereals used before the curry preparation. *Parmotrema* lichens are key ingredients in all popular garam Masala and briyani masala available in the Indian market, and also widely utilized in various dishes of south India.

(v) **Yangben or Limbu is the favorite dish of eastern Himalaya staple food culture:** Lichen *Parmelia* sp. is a type of edible lichen found attached to bark of chestnut and alder trees of Nepal. It is generally made by boiling the lichen with wood ash and consumed as dry curry. This particular lichen species is available only in the hill region of eastern Nepal and it is a favorite food of Limboo and Rai communities of Nepalis.

(vi) **Lichen salad:** Take a bowl and add black vinegar and equal volume of light soy sauce and appropriate amount of brown sugar. Mix the sugar until it dissolves completely. Add ginger, garlic, chilli flakes, sesame oil and mix well. Finally, add spring onions and allow it marinate for 10 minutes and then serve.

(vii) **Prather Ranch Beef Encrusted in Lichen:** Boil lichens *Parmotrema* for hours till the bitterness disappears. Drain the lichen and dehydrate overnight at 60°C unit it dries completely. Grind the dry lichen and dust it on a cylindrically cut tenderloin (beef) wrapped with foil. Cook at 60°C for 10 minutes in a water bath. Then cool it on an ice bath to make the lichen more flavourful. The beef is deeply fried in olive oil along with vegetables until it becomes brown. Sometimes red wine is also added while cooking. Finally, add few teaspoons of lemon, vinegar, and salt and transfer it to a baking sheet and put into an oven at 120°C until it gets a rosy pink color and then serve.

(viii) **Crispy lichens seasoned with dried egg yolks and very lightly cold-smoked fish:** First washed the lichen and boil at 100°C and deep fry. Then take out the fried lichen and keep in paper towel so that fat is sucked out. Then season the lichen over savoury cured egg yolks or very lightly cold-smoked fish and serve it crisp.

(ix) **Barbeque lichen:** This is made using *Alectoria* sp., commonly called Black lichen or 'Koonts' (meaning Hair). Usually it grows in pine and juniper trees. This black lichen is collected and soaked in water. Barbeque drum is set up by placing a rock inside the drum up to two feet and heated by fire. First place a stack of green pine over the rocks, then dry pine needles, followed by the soaked lichen and again a layer of dry pine and wet sack, and finally cover with soil. Allow the sealed drum to cook for 12 hours. The cooked large piece of black lichen is cut into small pieces and ground, dried and cooked with water along with little flour and sugar.

Lichens in Industrial Application

Dyes for Wool and Fabric

Many lichens are colored due to the presence of certain pigments such as usnic acid (yellow), pulvinic acid (yellow) and anthraquinones (red) which give a variety of colors to lichen. Lichens are usually light green to olive grey when they are moistened and wet; usually in the dry condition, it looks grayish to brown color. Making wool and fabric dyes from lichens is an ancient practice and is still continuing. Lichen specimens are collected, cut into small pieces and dipped in water containing ammonia. Earlier, urine was used as an alternative source for ammonia and the mixture was left for several weeks to obtain a dye (Shukla et al. 2014). The dye color obtained also varies with different lichen species soaked in water ammonia mixture and the type of extraction adopted to give out brown, pink, green, blue, and red color dye. Orchil is another popular dye extracted from lichen *Roccella* and *Leconara* used to dye woolen and silk fabrics. As we know, the lichens are slow growing and few species are in endangered list and overexploitation of lichen also affects biodiversity. Even modern fabric dyers emphasize the conservation of rare lichen forms and also gather specimen that have already detached from their substrate.

Litmus Paper

We all used litmus paper in our school days as acid-base indicator to know the exact pH of the substance. The mixture coated on the paper is nothing but a mixture of dyes obtained from lichens, especially *Roccella tinctoria*. Litmus papers are made using filter paper treating with lichen dye gives purple color at neutral pH. Moreover, it turns red when the litmus paper is exposed to an acid and blue at base.

Natural Sunscreens

Lichens are also known to absorb of ultraviolet radiation from the environment, especially *Xanthoria parietina.* This foliose lichen contains a yellow pigment called parietin which absorbs UV radiation and protects the algal cell from damage. It has been suggested that the lichen chemical parietin could be used as a natural sunscreen for humans.

Tanning

Few lichens are utilized in leather industry due to their astringent property. Especially lichen *Cetraria islandica* and *Lobaria pulmortaria* were processed and the astringent substance was extracted and utilized in the tanning industry.

Cosmetics and Perfumes

Lichens are also used as a fragrance substance since ancient times. The lichen *Ramalina, Roccella, Evernia*, and *Pseudorina* are found to have good fragrance due to the presence of perfumed volatile oils. The lichen thallus is processed and aromatic substances are collected and used in the preparation of cosmetics and perfumes.

Brewing and Distillation

Few lichen species such as *Cetraria islandica* contain higher quantities of carbohydrates in the form of lichenin. In Sweden and Russia, alcohol is produced by fermenting these lichens. These lichens are also used in confectionery.

Conclusions

The symbiotic lichen and their binding capacity are also effectual when they are combined during food preparation. Nowadays lichens have become an essential cheffy recipe for making exotic and creative dishes. However, still the popularity of lichen as food stuff is low which may be due to their indigestible nature. Lichens are rich in polysaccharide such as lignin and capable of producing certain acidic components like usnic acid, lecanoric acid, and atranorin which interfere with human metabolic activities. However, most of the lichens are edible when they are cooked and processed systematically. Among such dishes popular among various community people are Water gruel, Moss milk, Iceland moss tea, Yangben or Limbu, Prather Ranch Beef Encrusted in Lichen, Crispy lichens seasoned with dried egg yolks, Barbeque lichen, etc. Recently, the traditional food practice and the importance towards healthy diet have been drastically changing due to our modern life style. Creating awareness among people of the importance of traditional food, lichen identification, processing and their health benefits could possibly save us from health deterioration.

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6

Ethnic Probiotic Foods of South India and Their Health Benefits

Selvanathan Latha
Department of Petrochemical Technology, University College of Engineering, Bharathidasan Institute of Technology (BIT) Campus, Anna University, Tiruchirappalli, India

Anandhan Hemamalini
Centre for Excellence in Life Sciences, Bharathidasan University, Tiruchirappalli, India

Sundarraj Dinesh Kumar
Marine Planktonology & Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, India

Muthukumarasamy Arulmozhi
Department of Petrochemical Technology, University College of Engineering, Bharathidasan Institute of Technology (BIT) Campus, Anna University, Tiruchirappalli, India

Dharumadurai Dhanasekaran
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

1 What Are Probiotics?

The modern dietary practices together with stress, oldness, exposure to environmental toxins, uncontrolled hygienic practices and the overuse of antibiotics (both as medicine and food supplements) certainly lead to the imbalance of human gut microbiomes. The continuous use of antibiotics highly results in retention of antibiotic residues in tissues and generation of antibiotic resistant bacteria (Reid and Friendship 2002). Moreover, it is well known that rigorous use of antibiotics has negative effects not only on humans but also on food animals, which caused an increase in public and governmental interest in eliminating sub-therapeutic use of antibiotics. As a consequence, research has traditionally focused on finding alternatives to the application of antibiotics.

In recent years, "exploitation of live microbes as probiotics" is recommended as a promising alternative to antibiotics as prophylactic, therapeutic and growth promoting agents. They generally enhance the intestinal microflora by replenishing suppressed bacteria and inhibiting the growth of pathogenic flora. Probiotics are originally defined as "live microbial food supplements which beneficially affect the host by improving its intestinal microbial balance" (Fuller 1989). The word "probiotics" originated from the Greek words "pro" and "bios" which means "for life", which is opposed to "antibiotics" which means "against life". The term "probiotics" was first introduced by Kollath in 1953. They are also called functional food, colonic food, friendly bacteria, good or helpful bacteria, protective bacteria, and effective microbiota because they maintain a healthy gut. Till date several health benefits are claimed by the probiotic microbes. Some common conditions the probiotics treat are as follows:

- Irritable bowel syndrome
- Inflammatory bowel diseases
- Infectious diarrhea (caused by viruses, bacteria, or parasites)
- Diarrhea caused by antibiotics
- Atopic diseases like eczema
- Urogenital infections
- Allergies and colds
- Oral diseases
- Depression and anxiety

Probiotics have also exhibited antagonistic effects against pathogens belonging to the genera *Listeria, Clostridium, Salmonella, Shigella, Escherichia, Helicobacter*, and *Campylobacter*. The possible mechanisms underlying these antagonistic effects include competition for adhesion sites and nutritional sources, secretion of antimicrobial substances, toxin inactivation, and immune stimulation (Fooks et al. 1999).

2 Probiotic Microbes

Up to now, heaps of probiotic products have been introduced commercially with admirable health benefits. However, selection of suitable probiotic is the key to functionality and safety, since its effects are normally strain specific, dose specific, and condition specific. Moreover, determination of an optimal microbe for probiotic usage is completely empirical and some criteria must be considered for its selection (Figure 6.1).

The most expected characteristics include host origin, detailed definition and typing, survival under gastric and intestinal barriers, adherence and colonization to the intestinal epithelium, production of antimicrobial substances, safety, clinically proven beneficial health effects, viability during storage, etc. (Kolida et al. 2006). Furthermore, dead bacteria, products derived from bacteria, or end products of bacterial growth are not considered to be probiotics because they are not alive when administered to humans. Similarly, native bacteria are not probiotics until they are isolated, purified and proved to have health benefits.

So far, a variety of microbes have been frequently used as probiotics because of their proven health benefits (Table 6.1) which includes lactic acid bacteria (LAB) like *Lactobacillus* (*L. acidophilus, L. oris* and *L. plantarum*), *Bifidobacterium* (*B. animalis*, *B. bifidium* and *B. longum*), *Saccharomyces* (*S. boulardii* and *S. cerevisiae*), *Bacillus* (*B. subtilis* and *B. megaterium*), *Streptococcus* (*S. salivarius* subsp. *thermophilus*), *Enterococcus* (*E. faecalis* and *E. faecium*), *Candida* (*C. albicans*), and so on. LAB are Gram positive, non-sporulating and acid tolerant belonging to the phylum “Firmicutes”. They mainly encompass heterogeneous group from the microorganisms having a general metabolic property, i.e., production of lactic acid by anaerobic fermentation of carbohydrates. Although many bacterial genera produce lactic acid as a primary or secondary end product of carbohydrate fermentation, the term lactic acid bacteria is conventionally engaged for the genera in the order Lactobacillales that includes *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Amongst, strains of *Lactobacillus* are the most frequently used probiotics in humans.

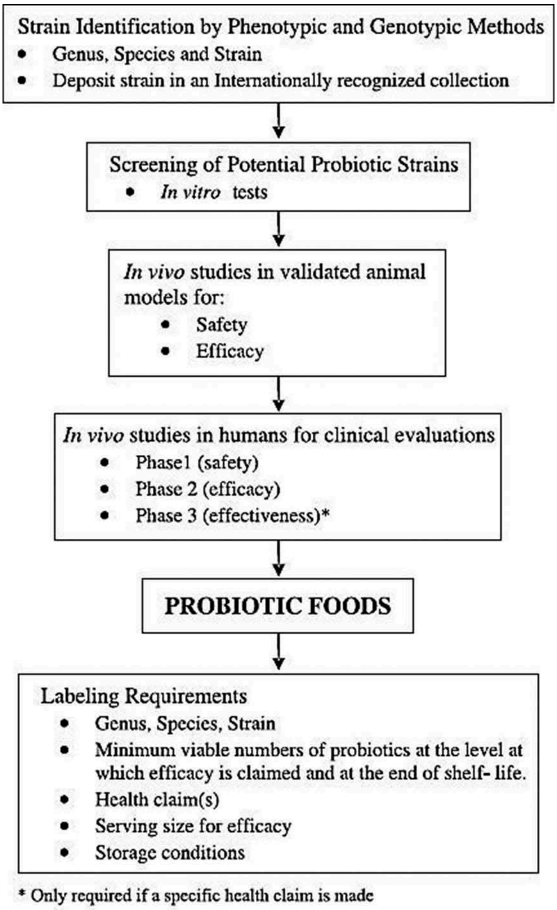

Strain Identification by Phenotypic and Genotypic Methods
Genus, Species and Strain
Deposit strain in an Internationally recognized collection
Screening of Potential Probiotic Strains
In vitro tests
In vivo studies in validated animal models for:
Safety
Efficacy
In vivo studies in humans for clinical evaluations
Phase1 (safety)
Phase 2 (efficacy)
Phase 3 (effectiveness)*
PROBIOTIC FOODS
Labeling Requirements
Genus, Species, Strain
Minimum viable numbers of probiotics at the level at which efficacy is claimed and at the end of shelf- life.
Health claim(s)
Serving size for efficacy
Storage conditions
* Only required if a specific health claim is made

FIGURE 6.1 Fundamental criteria for the selection of ideal probiotics.

Members of the genus *Bifidobacterium* are Gram positive, non-sporulating bacteria belonging to the phylum "Actinobacteria", which produce lactic acid as one of their major fermentation end products. Several *Bifidobacterium* strains are considered as decisive probionts and are used in food and health industries. Besides, they exert a series of beneficial health effects including regulation of intestinal microbial homeostasis, inhibition of bacterial pathogens colonizing the gut mucosa, modulation of local and systemic immune responses, repression of procarcinogenic enzymatic activities within the microbiota, production of vitamins and bioconversion of dietary compounds into bioactive molecules. In addition to LAB and bifidobacteria, certain Gram-negative bacteria, fungi (yeasts and moulds), bacteriophages, cyanobacteria and unicellular algae have potential probiotic properties (Das et al. 2008). The commercial probiotics exist either in the form of

- Powder
- Liquid
- Gel
- Paste
- Capsules
- Sachets or
- Granules

TABLE 6.1

List of commonly used probiotics in human health improvement

S. No.	Class and family	Genus	Species and subspecies
1.	Bacilli and Lactobacillaceae	*Lactobacillus*	*acidophilus* *casei* *crispatus* *fermentum* *gallinarum* *gasseri* *helveticus* *johnsonii* *oris* *plantarum* *reuteri* *rhamnosus* *salivarius*
2.	Bacilli and Lactobacillaceae	*Pediococcus*	*acidilactici*
3.	Bacilli and Leuconostocaceae	*Leuconostoc*	*mesenteroides*
4.	Bacilli and Leuconostocaceae	*Weissella*	*cibaria* *confusa*
5.	Bacilli and Streptococcaceae	*Lactococcus*	*lactis* subsp. *cremoris* *lactis* subsp. *lactis*
6.	Bacilli and Streptococcaceae	*Streptococcus*	*salivarius* subsp. *thermophilus*
7.	Bacilli and Enterococcaceae	*Enterococcus*	*faecalis* *faecium*
8.	Actinobacteria and Bifidobacteriaceae	*Bifidobacterium*	*adolescentes* *animalis* *bifidum* *breve* *infantis* *longum*
9.	Actinobacteria and Propionibacteriaceae	*Propionibacterium*	*freudenreichii*
10.	Saccharomycetes and Saccharomycetaceae	*Saccharomyces*	*boulardii* *cerevisiae*

The marketable probiotic may be made out of a single bacterial strain or it may be a consortium as well. In some cases, probiotics formulated together with prebiotic substances (dietary fibers that help feed the friendly bacteria already present in the gut). Some of the commercially available single and multi-strain probiotics are listed in Tables 6.2a and 6.2b.

TABLE 6.2A

Commercially available probiotic preparations in Indian market—single strain probiotics

S. No.	Products	Composition
1.	Cynobac Tender/Baclac/Lactra/Rexflora kid tab/Oraflora	*Lactobacillus sporogenes* (60 million spores/Tab)
2.	Cynobac/Rexflora Cap	*Lactobacillus sporogenes* (180 million spores/ Tab or Cap)
3.	Econorm/Biogit/Stibs	*Saccharomyces boulardii* (250 mg/Sachet)
4.	Enterogermina	*Bacillus clausii* (200 million spores/5 ml)
5.	Laviest	*Saccharomyces cerevisiae P* (10 million cells/Cap)

TABLE 6.2B

Commercially available probiotic preparations in Indian market—multi-strain probiotics

S. No.	Products	Composition
1.	Bifilac	*Lactobacillus sporogenes* (50 million spores), *Streptococcus fecalis* (30 million), *Clostridium butyricum* (2 million) and *Bacillus mesentricus* (1 million)
2.	Flora-SB	*Lactobacillus acidophilus* (75 mg), *Lactobacillus rhamnosus* (75 mg), *Bifidobacterium longum* (75 mg), *Bifidobacterium bifidum* (50 mg) and *Saccharomyces boulardii* (50 mg)
3.	Lactisyn	*Lactobacillus lactis* (490 million), *Lactobacillus acidophilus* (490 million spores), *Streptococcus thermophilus* (10 million) and *Streptococcus lactis* (10 million)
4.	Eugi	*Lactobacillus acidophilus* (240 million), *Lactobacillus rhamnosus* (240 million), *Bifidobacterium longum* (240 million), *Bifidobacterium bifidum* (240 million), *Saccharomyces boulardii* (50 million), *Streptococcus thermophilus* (240 million) and Fructo-oligosaccharides (300 mg)
5.	Prowell	*Lactobacillus acidophilus* (5000 CFU), *Bifidobacterium bifidum* (5000 CFU), *Bifidobacterium longum* (5000 CFU), *Bifidobacterium infantis* (5000 CFU) and Fructo-oligosaccharides
6.	Biogut	*Lactobacillus acidophilus* (300 million), *Lactobacillus rhamnosus* (300 million), *Lactobacillus paracasei* (300 million), *Lactobacillus sporogenes* (1200 million), *Bifidobacterium longum* (300 million), *Saccharomyces boulardii* (600 million), Inulin (200 mg) and Fructo-oligosaccharides (200 mg)
7.	Bifilin	*Lactobacillus acidophilus* (500 million), *Lactobacillus rhamnosus* (500 million), *Bifidobacterium bifidum* (500 million), *Bifidobacterium longum* (500 million), *Streptococcus thermophilus* (250 million), *Saccharomyces boulardii* (250 million), Fungal amylase (200 mg) and Fructo-oligosaccharides (100 mg)
8.	Binifit	*Streptococcus fecalis* (30 million), *Clostridium butyricum* (2 million), *Bacillus mesentricus* JPC (1 million) and *Lactobacillus sporogenes* (50 million)
9.	Ecoflora	*Lactobacillus rhamnosus* GR-1 (1000 million CFU) and *Lactobacillus reuteri* RC-14
10.	Flora-SB	*Lactobacillus acidophilus* (40 mg), *Lactobacillus rhamnosus* (40 mg), *Bifidobacterium bifidum* (25 mg), *Bifidobacterium longum* (40 mg), *Saccharomyces boulardii* (25 mg) and Fructo-oligosaccharides (100 mg)
11.	Diacowin	*Saccharomyces boulardii* (141.25 mg) and Lactic acid bacilli (50 million)

Indigenous probiotic microorganisms are commonly isolated from human and animal intestinal tracts. However, other isolation sources like fermented food products wherein LAB are used as starter cultures or occur spontaneously as the fermentative microbiome may offer potential probiotic organisms as well (Mathara et al. 2008; Garai-Ibabe et al. 2010; Abriouel et al. 2012; Saad et al. 2013).

3 Fermented Probiotic Foods

Fermented foods are consumed throughout the world and in some countries they have played significant roles in the systematic diet for centuries. They have frequently been produced for preserving perishable primary products such as milk and meat, where other possibilities like refrigeration or pasteurization were unavailable or too expensive. So, the fermentation process used for the preparation of foods is always indispensable and has universal appeal. This process not only preserves the foods but also boosts up their characteristics like shelf-life, texture, palatability, digestibility, nutritive value, safety and other functional properties of foods and beverages (Jeyaram et al. 2009). It mainly results in the breakdown of non-digestible carbohydrates; improved availability of bioactive molecules, essential amino acids, vitamins and minerals, and enhancement of overall quality and aroma of food, and thereby making them more desirable to the consumer.

At present, more than 5000 types of fermented foods with different base materials are manufactured worldwide, many of which are traditional. They are typically produced in the household or in cottage industry using relatively simple techniques and equipment (Aidoo et al. 2006). In India, such fermented foods are largely prepared from milk, vegetables and the most common cereals including rice, corn, wheat, millet and sorghum, and their preparation remains a culinary skill. Most of the traditional south Indian dishes are a rich source of probiotic microbes. More recently, the consumption of such fermented probiotic foods has emerged as an important dietary strategy for improving human health (Marco et al. 2017) as they contribute a diverse array of live microorganisms to the existing gut microbiota. Over many years, humans identified that bacteria and other microbes existing in fermented foods are capable of transforming food substrates, making them both tasty and nutritious. Mainly, the LAB and yeasts play a significant role in various natural food fermentations like cheese, curd, pickles, and many other traditional foods. Besides, they are closely linked with the human environment. The lactic acid bacteria associated with fermented foods include species of the genera *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus*, and *Weissella* (Stiles and Holzapfel 1997; Iyer et al. 2011). The number of organisms existing in the fermented foods varies significantly as they depend on how products are manufactured and processed, as well as conditions and duration of storage.

Lactic acid fermentation processes are ancient and the most important economical forms of production and preservation of foods for human consumption. They comprise of chemical changes in foods accelerated by enzymes of lactic acid bacteria resulting in a variety of fermented foods. Homo- and hetero-fermentative LAB are commonly fastidious on artificial growth media; however, they propagate readily in most of the food substrates and lower the pH rapidly to a certain point in which other competing microbes are no longer able to grow. Furthermore, LAB are dynamic residents of human intestinal ecosystem and have a wide range of application as probiotic organisms for rendering multifactorial health benefits to humans and other animals. Mainly, they exhibit antimicrobial activity against several Gram-positive and Gram-negative pathogens while this activity is rare against fungi (Schnurer and Magnusson 2005). The antibacterial effect of LAB is primarily due to fermentation and is attributed to organic acids, bacteriocins, hydrogen peroxide, diacetyl, low pH, competition and nutrient depletion, altered redox potentials, stimulation of the immune system, and deconjugation of bile acids (Satish Kumar et al. 2010). Studies have also confirmed the correlation between oral administration of *Lactobacillus* or *Bifidobacterium* species and their important role in luminal oxalate reduction, which decreased the risk of urinary oxalate excretion in humans and animals. Hence, the traditional uses of various LAB as fermentation agents for foods are considered to be safe for the general population. Some of the lactic acid bacteria-mediated fermented foods are categorized as follows: cereal-based traditional probiotic foods, milk-based traditional fermented probiotic foods, and vegetables- and fruits-based traditional fermented probiotic foods and beverages.

4 Cereal-Based Traditional Probiotic Foods and Beverages

In the modernistic world, overconsumption of fast foods, highly processed foods and junk foods leads to adverse health conditions and is the cause of many diseases (Kearney 2010). According to the human epidemiological studies, food intake with high calorific value causes about 90% of the diseases, including chronic metabolic diseases such as diabetes, cardiovascular diseases, obesity, colon cancer, ageing, etc. Therefore, disease control via dietary modulation is desirable for the present fast food world. In this scenario, cereals are recognized as the best choice for low calorie diet because they act as the most significant source containing essential dietary proteins, energy, vitamins, minerals, carbohydrates, oligosaccharides and fiber. These nutritionally rich products deliver recommended nutrients to the human health. Furthermore, the vast distribution and high nutritive value of cereals makes them as effective raw materials for the development of new non-dairy, fermented functional foods.

The Indian history substantiated the utilization of distinctive cereal grains by our ancestors, and also the ingestion of grains in different civilizations of India can be witnessed from the literatures. These cereals and legumes are grown in India since the Indus valley civilization (9000–5500 BC) period (Samanta et al. 2011). The major cereal crops grown worldwide include rice (*Oryza sativa*), wheat (*Triticum aestivum L.*), maize/corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.). Amongst this, rice is widely used in the southern region of India, particularly in Tamil Nadu whereas wheat is used in northern India for making breads and “rotis”. Other cereals include millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*), rye (*Secale cereale*), oat (*Avena sativa*) and triticale (*Triticale hexaploide*) in which oats and barley mainly contain beta glucan, a major functional component of cereal fiber. Research has evidenced the reduction in the risk of cardiovascular diseases due to the hypocholesterolemic effect of this compound (Angelov et al. 2006). Similarly, the three pulses namely, urad (*Vigna mungo*), mung (*Vigna radiata*), and masoor (*Lens culinaris*) are the principal legumes used in the preparation of a variety of fermented foods in different parts of the world.

Cereal- and legume-based mixed fermented food products have complementary nutritional value and the traditional methods used for fermenting cereals and legumes are simple and inexpensive. Almost all the cereal grains have high calorific value, mostly from starch and proteins, and thus received attention due to their prospective health benefits and functional properties. Some of the grains unveil anti-nutritional properties like the presence of trypsin inhibitors; however, the traditional processing techniques such as fermentation, soaking and cooking remove those factors and help to improve the utilization of nutrients. Nowadays, cereals have become the main focus of health care industries wherein the traditionally processed (germinated and fermented) and non-processed (non-germinated and non-fermented) cereals are used in the preparation of foods. Cereal products often ferment rapidly resulting in enhanced shelf life and nutritional properties compared with the raw materials (Hassan et al. 2012). Generally, single or mixed cereals are used as an effective substrate in the making of probiotic-incorporated functional foods and the final products can differ according to the fermentation conditions and the microbial population. Several researchers (Pal et al. 2005; Angelov et al. 2006) have worked on cereal- and legumes-based food products that increased their nutritional quality by fermentation using LAB and other probiotic microbes.

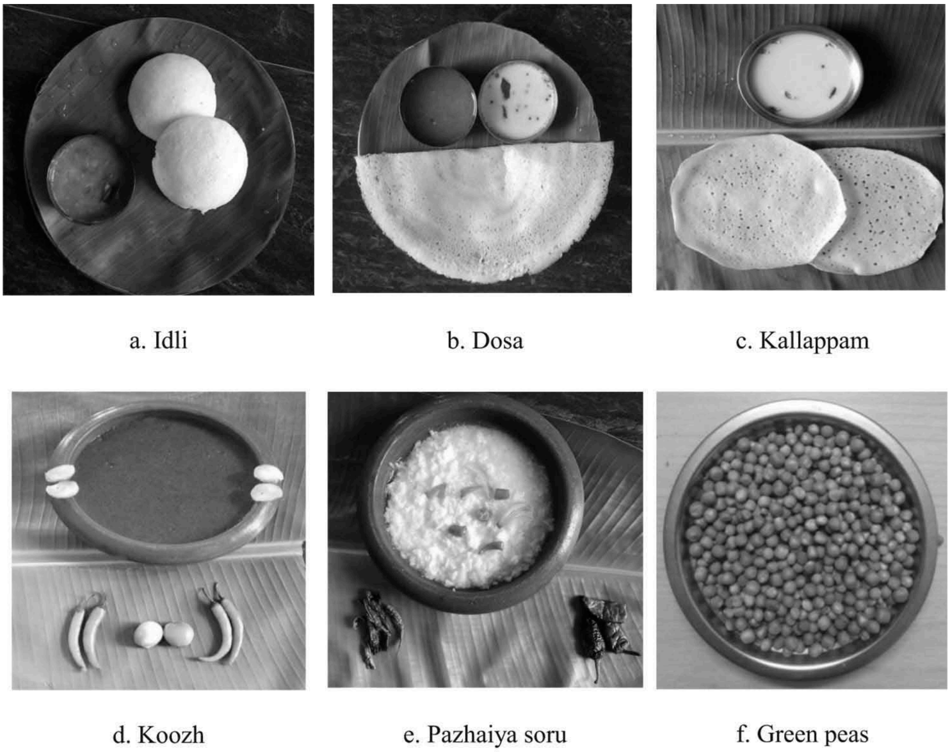
Fermentation of cereals by LAB such as *L. acidophilus, L. plantarum,L. casei* and *L. reuteri* reveals several advantages including general improvement in shelf life, taste, smell, texture, nutritional value, digestion of proteins and finally increases the organoleptic qualities. They mainly contribute to the degradation of phytic acid that binds divalent cations like zinc, iron and calcium, and decreases their bioavailability. Degradation of phytic acid is caused by the enzyme phytases that are present in cereals and are produced by LAB (El-Aidie et al. 2017). The lactose intolerance, allergenic to milk proteins, high cholesterol content, and economical reasons which are associated with dairy products along with the vegetarian tendencies of diverse populations, tend to enforce the recent recourse to non-dairy products such as cereals, fruits/vegetables or others as probiotic carriers (Gobbetti et al. 2010; Enujiugha and Badejo 2017). Among these, cereals fulfill the prebiotic criteria and also increase the beneficial microbial growth in human digestive system due to their soluble and insoluble fiber contents. Moreover, cereal grains act as the richest source of phytochemicals, phenolic compounds and substances with antioxidant activity, and hence they can be used as most suitable vehicles for probiotic delivery. Most of the cereal foods (with/without pulses) such as idli, dosa, koozh, dhokla, nan, parotta, ambali, and pazhaiya soru are consumed on a daily basis by the local population. Some of the traditional cereal-based probiotic foods with their functional and health profits are discussed below.

4.1 Idli

Idli or idly (low-caloric starchy foodstuff) is a steamed, white colored, small, round, and spongy product, originating from the Indian subcontinent. It is a perfect healthy, versatile and all season breakfast food in southern India and northern Sri Lanka. Idli with hot sambhar and a variety of tasty chutneys (tomato, tamarind and coconut) for breakfast is a relatively very common delicious food in the south Indian households (Figure 6.2a). There are many varieties of idlis made in India including quick rava idli, oats idli, ragi idli, dahi idli, varagu idli, barley idli, paneer vegetable idli, stuffed potato idli, kanchipuram idli, butter idli, fried idli, and so on. For the last three years, March 30 has been celebrated as "World Idli Day", the idea being the brainchild of M. Eniyavan, founder of Mallipoo Idly, a popular chain in Tamil Nadu.

Idli is typically prepared by steaming a fermented batter comprising of rice (*Oryza sativa*), de-husked black gram or lentils (*Vigna mungo*), salt, fenugreek seeds and water. Amongst this, black gram is the prime component having naturally fermenting microbes and acts as a substrate for the batter fermentation. During the preparation of batter, rice and black gram are used in the ratio of 2:1, wherein soybean (*Glycine max*), green gram (*Vigna radiata*) and chick pea (*Cicer arietinum*) can be substituted for the black gram. By undergoing efficient fermentation by a mixture of natural microbiomes, the bio-availability of its nutritional efficiency value, especially proteins and minerals, increases which helps the body to assimilate more nutrition.

Leavening is the key objective of fermentation in making of savory cake idli where the batter is kept overnight at room temperature for effectual fermentation. The major microorganisms involved in the leavening process are LAB including *Leuconostoc mesenteroides, L. delbrueckii,*


a. Idli b. Dosa c. Kallappam

d. Koozh e. Pazhaiya soru f. Green peas

FIGURE 6.2 Cereal-based functional foods and beverages.

L. fermentum, L. coryneformis, L. plantarum, L. lactis and *Streptococcus faecalis*, and other microbes such as *Saccharomyces cerevisiae, Pediococcus cerevisiae, Debaryomyces hansenii, Hansenula anomala, Torulopsis candida* and *Trichosporon beigelii* (Sridevi et al. 2010; Satish Kumar et al. 2013). Each plain idli has approximately 39 calories, 8 grams carbohydrates, 2 grams dietary fiber, 2 grams protein and no fat. Fermentation practice improves the batter volume, soluble solids, total acids, level of vital enzymes (amylase and protease), essential amino acids (lysine, cysteine and methionine), soluble vitamins (folate, vitamin A, B1, B2 and B12), non-protein nitrogen content with a persistent reduction in anti-nutrient phytic acid, which boosts the probiotic action in human health (Moktan et al. 2011).

Due to the fermentation process, idli is more digestible and thus it is suggested to all age groups. It is largely considered as anti-obesity and weight loosing diet, therefore useful to decrease the risk of stroke, high blood pressure and cardiovascular diseases. Furthermore, idli is used as a dietary supplement to treat children suffering from protein calorie malnutrition and kwashiorkor. The micronutrients such as iron, zinc, folate and calcium prevent anemia, and also enable the oxygenation of blood and nourishment of the muscle and bone. The carbohydrate as well as dietary fiber content stimulates healthy digestion, bowel movements, and formation of soft and bulky stool.

4.2 Dosa

Dosa is a crispy, flat thin pancake prepared from the fermented rice batter and black gram or lentils (Figure 6.2b). It is one of the most popular staple foods of southern India, and has a long history though not all detail can be evidently outlined. Dosa is consumed regularly as breakfast, dinner or sometimes as snacks since it is a highly seasoned pancake. It is generally made on a hot griddle (tava) greased with oil and served with different types of chutneys prepared from tomato, tamarind, coconut and sambhar which is made by adding vegetables to red gram dhal. There are many varieties of dosa made in India, which includes masala dosa, onion dosa, rava dosa, ragi dosa, adai dosa, onion rava dosa, ghee roast dosa, set dosa, neer dosa, egg dosa, chicken dosa, oats dosa, wheat dosa, moon dal dosa, cheese dosa, paper dosa, bread dosa, jini dosa, benne dosa, poha dosa, schezwan dosa, sada dosa, curd dosa, butter rava dosa, ural dal dosa, vendhaya dosa, soybean dosa, etc.

Dosa is a low calorie food holding a good quantity of intestinal bacteria and thus considered as a healthy breakfast. Each plain dosa comprises ~120 calories with 2 grams protein, 1 gram dietary fiber, 17 grams carbohydrates and 3 grams fat. Dosa is more digestible and nutritionally dense, and hence it is suggested to elderly people and children younger than 10 years. On top of that, dosa is considered as a suitable vegan diet for people with wheat allergies or gluten intolerance. Its low glycemic content and glycemic index mainly help to fight against pre- and post-diabetic conditions. Further, dosa provides sufficient energy for prolonged physical endurance, and also it is considered as an effective therapeutic agent for rheumatism and neural disorders. It reduces the anti-nutrients and thereby increases the bio-accessibility of zinc and iron. Dosa increases the absorption of essential minerals from gastrointestinal tract, hence preventing from the mineral deficiencies. It is also believed that dosa can be used to improve fertility, foetus weight and breast milk.

The microbiological, physical and biochemical changes of dosa during fermentation and its nutritive values are quite similar to idli. Lactic acid bacteria are the predominant group of microbes participating in the fermentation of *dosa* that includes *L. mesenteroides, Streptococcus faecalis, Bacillus amyloliquefaciens, Torulopsis candida, L. fermentum*, *L. lactis, L. delbrueckii* and *L. plantarum*. Alike the yeasts belonging to *Saccharomyces cerevisiae, Debaryomyces hansenii, Trichosporon beigelli*, *Torulopsis* sp. and *Trichosporon pullulans* are the minor groups associated with batter fermentation. Dosa fermentation enhances the total batter volume and the amount of total solids, total acids, non-protein nitrogen, free amino acids, amylases, proteinases, vitamins B1 and B2, folic acid, amino nitrogen, formation of diols, antimicrobial and antioxidant substances.

Due to the bacteriocin (antibacterial protein) producing ability, LAB derived from dosa inhibited the pathogens such as *B. cereus, Staphylococcus aureus, Listeria monocytogenes,* *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Pal et al. 2005). Gupta and Tiwari (2015) also reported similar antibacterial effect for the bacteriocin of a probiotic *Enterococcus hirae* LD3, an isolate of dosa batter that hinders the growth of both Gram-positive and Gram-negative groups, including *M. luteus*, *L. monocytogenes*, *S. typhi*, *S. aureus*, *S. flexneri*, *P. fluorescens*, *P. aeruginosa*, *E. coli* O157:H7 and *Vibrio* sp.

4.3 Kallappam

Appam or hopper, specifically kallappam, is a delicious and nutritious fermented rice pancake. It is a traditional breakfast food in south Indian cuisine exclusively in Tamil Nadu, Kerala and Sri Lanka. Kallappam is super soft, fluffy and white in color with delicate texture (Figure 6.2c). Its tastes almost similar to the lacy edged palappam and can be eaten with any type of stew or spicy veg/non-veg curries. Normally, kallappam is made with rice batter and coconut milk and has additional ingredients such as cumin and shallots. The name kallappam originated from the word “Kallu” which means “Toddy” (local mild alcoholic drink or local beer) and it is used as the raising agent during fermentation. Hence, kallappam is also known as “Toddy Appam” or “Toddy Bread”. During the preparation of kallappam, fermented sweet toddy is added to the rice batter as an additional source of LAB such as *Weissella paramesenteroides, L. plantarum* and *L. fermentum*, which gives a unique taste (Satish Kumar et al. 2013). But, its closest substitute yeast is used nowadays due to the unavailability of toddy. Besides, the fermented batter is prepared either as steamed cakes (idli) or as pan cakes (dosa and appam) before it becomes too soured.

Kallappam might also denote appam cooked on a griddle (kal) instead of appachatti (curved pan). However, sometimes it is confused with vellayappam or vattayappam which is a sweeter and steamed delicacy popular in Kerala. Each kallappam contains 120 calories with 20 grams of carbohydrates, 4.1 grams of dietary fiber, 2.7 grams of sugar, and 10 grams of fat. Kallappam has several medicinal values including antimicrobial potentials. Satish Kumar et al. (2011) and (2012) reported that *L. plantarum* AS1, an isolate of kallappam, had antipathogenic and anticancer effects which is substantiated by the prevention of *V. parahaemolyticus* (entero-virulent bacterium) colonization in HT-29 cell lines and colorectal cancer in male Wistar rats.

4.4 Koozh

Koozh (the Tamil term for porridge) is a traditional south Indian weaning food largely consumed in rural and urban households. It is comes under the category of ready to eat (RTE) foods or energy beverages. Koozh is a soft food prepared in a mud pot (Figure 6.2d) from processed (germinated and fermented) millet, either from kezhvaragu/ragi (finger millet/*Eleusine coracana* L.) or kampu (pearl millet/*Pennisetumg laucum* L.) flour and broken rice (called noyee in Tamil) (Satish Kumar et al. 2010). Amongst, finger millet koozh serves as an ideal low calorie diet for all age groups especially growing infants and pregnant women due to its rich source of carbohydrate, protein, dietary fiber, amino acids and phytochemicals. It also contains minerals such as calcium, magnesium, phosphorous and manganese that are indispensable for normal growth of body tissue and energy metabolism (Chethan et al. 2008).

Koozh is consumed by the rural populations as a breakfast as well as offered in temples during the time of festival. In south India, koozh is sold in the streets or by mobile food vendors at the time of summer since it is easily digestible and cools down the body. Koozh is unique food wherein fermentation occurs twice—before and after cooking, so its preparation last for two days. It has short shelf life and so is normally drunk within 12 hours of its preparation. Conventionally, koozh is deliberated as nutritious and health promoting, but there is very few scientific documentation on its nutritive and microbial composition. Satish Kumar et al. (2010) reported the isolation and probiotic characteristics of lactic acid bacteria *W. paramesenteroides* and *L. fermentum* in koozh.

4.5 Pazhaya Soru

Pazhaya soru or kanji is another fermented dish largely used as a staple diet in the south Indian region particularly in Tamil Nadu and Kerala during the hot summer days. This fermented rice porridge is also known as “pazhaya saadham” in Tamil, “cold rice” in English, “vellachor” in Malayalam and “saddhi annamu” in Telugu. It is a healthy and nutritious dish made using leftover steamed rice wherein the rice is added with water and undergoes overnight incubation at room temperature in a mud pot or earthen vessel (Figure 6.2e). In next morning, it is directly consumed by mixing buttermilk and salt. Pazhaya soru can be combined with either mor milagai (sun-dried chillies), karuvadu (dried fish), non-spicy pickles (goose berry or lemon), slightly salted raw onions and green chillies (Sekar and Mariappan 2007).

Pazhaya soru and the water drained from the fermented rice called “neeragaram” (left over rice porridge) are the good early morning diet for farmers prior going to the agricultural field. This diet keeps the villagers and farmers energized throughout the day and also cools down the body from the scorching sun during their farming work. Consuming this rice porridge also takes away our body’s craving for tea or coffee. Thus, it is found out that pazhaya soru is a very compact and complete breakfast. Moreover, food scientists who investigated on the food practices among various countries of the world concluded that the South Asia’s tradition of consuming pazhaya soru is the best breakfast.

The slightly fermented rice porridge has lots of health benefits. This rice generates and harbours trillions of beneficial bacteria that aid in quicker digestion, and has several disease fighting and immunity developing agents. The lactic acid bacteria present in the porridge break down the anti-nutritional factors in rice resulting in an improved bio-availability of micro-nutrients and minerals such as potassium, iron and calcium. Further, the bacteria which proliferate in the intestines due to this rice defend the internal organs and retain all organs battle fit and ready. It is believed that frequent intake of kanji can comfort from stomach ulcers and prevents from dehydration during summer seasons. Consuming the rice porridge heals bone-related ailments and muscular pains and wards off ageing. Pazhaya soru is also rich in rare vitamins, especially vitamins B6 and B12 that are not easily accessible in other food supplements.

Brown rice is the best choice for the preparation of this fermented rice as its nutrients are retained intact. The American Nutrition Association has listed the following health benefits for people who strictly practice intake of fermented rice porridge.

- Consuming this rice as breakfast keeps the body light and also energetic.
- Beneficial bacteria for the body get produced in abundance.
- Blood pressure is normalized and hypertension subsides appreciably.
- As this food is very fibrous, it removes constipation and also dullness in the body.
- It removes all types of ulcers in the body.
- Fresh infections are kept at bay due to consuming this rice.
- It helps in maintaining youthful and radiant look.
- This removes allergy-induced problems and also skin-related ailments.
- Stomach ailments disappear when this is consumed in the morning as excessive and harmful heat retained in the body is neutralized.
- The body feels less tired due to this food as a result of which one feels fresh throughout the day.

4.6 Green Peas

Green peas are a starchy legume with all kinds of health benefits, and it mainly fosters a favorable gut balance (Figure 6.2f). Raw peas actually contain a specific bacterium *L. mesenteroides*, a strain of inherent probiotic commonly found in fermented foods. It has high antibodies (IgA)-inducing ability that supports the immune system in particular. Due to the presence of potent probiotic, green peas may also be able to fight off infections and colds by protecting the mucosal barrier which defends bad bugs and removes toxins from the body. *L. mesenteroides* is often associated with fermentation under very low temperature conditions and cannot survive at high temperatures; therefore, it is better to consume raw green peas to fill the gastrointestinal tract with good bacteria. Lacto-fermented green peas appeared a lot like the canned or cooked stuff with analogous texture and color, which means that normal fermentation yields similar results as cooking confiscates the starchy bite and makes peas more tender. The foremost advantage is that they provide additional dose of vitamins B and C, by-products of microbial metabolism and an assortment of live probiotic cultures which make our body more resilient to disease and stress conditions.

5 Milk-Based Traditional Fermented Probiotic Foods

Milk and milk-based fermented food products are consumed most popularly in India. Majority of foods in this category are prepared by simply adding lactic acid bacteria into the milk of cow, buffalo or yak and allowed to ferment for a particular period. It is easily curdled by pathogenic microorganisms and hence fermentation of milk using lactic acid bacteria is preferred for the prevention of nutritional quality. They mainly convert milk sugar lactose into lactic acid and selective strains produce antibacterial substance bacteriocin to suppress milk curdling bacteria.

5.1 Curd

Curd or Dahi is a soft, thick, white food substance which is formed by coagulating the milk (Figure 6.3a). It is excessively used as a dairy product all over the world and acts as the basis for cheese preparation. Curd is recognized as one of the best easily accessible natural probiotic foods which account nearly 90% of the total production of fermented milk foodstuffs in India (Behare et al. 2009). The history of curd is found in texts as old as 700 B.C. and it has been prevalent since Vedic times. It is largely specified in ancient scriptures such as Vedas, Upanishads and various hymns. During Lord Krishna's time (ca. 3000 B.C.), curd, buttermilk and country butter were greatly regarded. Ayurveda, the traditional scientific system of Indian medicine, also highlights the therapeutic characteristics of cow and buffalo milk curd.

Curd is extremely prevalent with consumers because of its characteristic odour and flavor, and it is also recommended by numerous dieticians. It is typically prepared from the milk of cow and buffalo. Moreover, the milk of yak and zomo are also used in the preparation of curd in the Himalayas. Up till now, curd is traditionally made in homes and restaurants as well as by local halwais and shops. Few dairies have also started its commercial manufacture in the Indian region. Curd is generally used with different forms in the culinary preparations of Indian people, particularly consumed with rice in South India and with wheat in the north. It is also utilized as a beverage or dessert that can be added to our regular diet. Curd can be eaten alone or either with chopped raw onions, tomatoes and antioxidant-rich fruits such as apples, blueberries and strawberries.

Curd is relatively similar to plain yogurt in its appearance as well as consistency, but mainly differs in its use of mixed starters of mesophilic lactococci. Curd mostly comprises a mixed culture of *Streptococcus* and *Lactobacillus* species in addition to the yoghurt organisms such as *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (Vijayendra et al. 2008; Behare et al. 2009). Moreover, curd contains the foremost flavor-inducing metabolite diacetyl which is more appreciated by the people of South Asian origin when compared to the acetaldehyde flavor of yogurt (Yadav et al. 2007).

Curd is very light in nature and easier to digest than milk. Including a bowl of curd in every day lunch could sustain a healthy gut flora of humans. It is believed to have good nutritional and

a. Curd b. Buttermilk

FIGURE 6.3 Milk-based fermented probiotic foods.

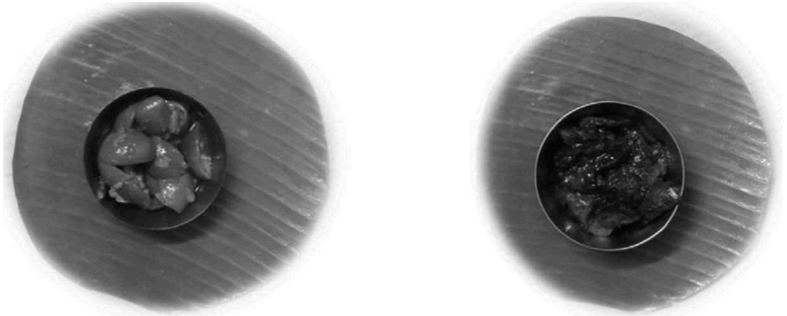
therapeutic values and thus gained widespread consumer acceptance as a healthy food. Curd is loaded with many essential nutrients like calcium, vitamin B2 and B12, potassium and magnesium. Curd is deliberated as a powerful probiotic that has a good moisturizing effect on our skin. It can also be used as one of the best homemade solutions in getting rid of dandruff as it having lactic acid, which is anti-fungal in nature.

5.2 Buttermilk

Buttermilk is the liquid that is left over when butter is churned out of cream, consumed with or without added salt and spices (Figure 6.3b). It has less fat content and fewer calories compared with regular milk or dahi. Buttermilk is as rich a source of calcium and protein as milk. In the states of Gujarat and Rajasthan of India, buttermilk is also called chaach. It is easier to digest and with the presence of live cultures, helps in intestinal health. Buttermilk is consumed with meals and is suggested in the Ayurvedic medicinal system (Pushpangadan et al. 2012).

6 Vegetables and Fruits–Based Traditional Fermented Probiotic Foods and Beverages

Fresh fruits and vegetables are more difficult to store. It seems that the preparation of fermented fruit and vegetable products was introduced by the ancient people who started collecting and storing food. Fresh fruits are normally rich in juices and sugars and are slightly acidic in nature. These constituents induce the growth of yeasts and are certainly used for making alcoholic beverages. Besides, fruits are an ideal medium for the functional foods, and have more nutritional values and impact on human health due to the presence of various phytochemicals, antioxidant contents, flavonoids, carotenoids, no cholesterol, vitamins, minerals, electrolytes, dietary fibers and other bioactive compounds (Yoon et al. 2004). Fruits are healthy, refreshing, and have good taste and flavor profile which makes them more suitable for probiotics. The highly perishable and short shelf life of fruits necessitates for immediate processing to reduce the post-harvest losses, and probiotic product development can be an approach to enhance the availability and market value of a product (Panghal et al. 2017). Dairy allergens are also preventing some people from consuming dairy probiotics, and fruit and vegetables are free from dairy allergens, lactose, and cholesterol and so are a suitable substrate (Luckow and Delahunty 2004). Fruit-based probiotic products are made by pineapple, cranberry, strawberry, sweet lime, mango, grapes, cashew apple, olive, oranges, and so on.



a. Lemon pickle b. Mango pickle

FIGURE 6.4 Vegetables-based probiotic foods.

Similar to fruits, fresh vegetables comprise vitamins, mineral contents, carbohydrates, and health promoting compounds that are utilized to build up body and well for its restoration (Yadav et al. 2014). Also, they are rich in phytochemicals and phytonutrients, and these vegetables are most suitable for the lactose-intolerant people due to the absence of lactose. The vegetables cabbage, tomato, carrot, beet root, onion, ginger, peanuts, etc. are used in probiotics. The lactic acid bacteria like *L. acidophilus, L. plantarium, L. casei* and *B. longum* are mostly utilized for vegetables-based probiotic foods wherein the lactic acid fermentation increases the nutritional value of products. Some of the traditional fermented health foods are developed in India using fruits and vegetables, and their health impacts are discussed in what follows.

6.1 Pickles

Pickles are another classic fermented foods for vegetarians and they have been popular in Asia for centuries. They are generally prepared by fermenting the vegetables and special fruits. The vegetables pickled include mango, lemon, carrot, cucumber, cauliflower and onion (Figure 6.4a, b). Pickles can be easily formulated at home or in small factories within a short period of time and can also be purchased from the grocery shops. But they might lack the natural enzymes which usually become demolished during the process of their preparation. Fermented vegetables are rich in probiotics and are used as appetizers and served with almost every meal. Cucumber is one of the oldest vegetables constantly cultivated by people which are thought to have originated in India more than 3000 years ago. It is utilized both as a fresh vegetable and a pickled product.

7 Conclusions

Fermentation technique is one of the oldest and most economical methods, primarily aimed for food production and preservation. It has also been explored for other functions such as improvement in food safety through inhibition of pathogens or removal of toxic compounds, improvement in nutritional value as well as organoleptic quality of the food. There is so much diversity in traditional fermented foods of India because the regional health foods have evolved according to the climate, culture and cropping practices of a particular region. Moreover, certain foods have become more popular in certain regions according to the health condition of a population. They have long been a part of the human diet, and with further supplementation of probiotic microbes, they offer nutritional and health attributes worthy of recommendation of regular consumption.

The expansive use of fermented foods also supports their greater inclusion in Food Guides around the world, and the knowledge of kind and key roles of the probiotic microbes in fermented foods would facilitate the development of starter cultures for fruitful fermentation.

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7

Ethnic Selected Fermented Foods of Greece

Antonia Terpou
Department of Chemistry, University of Patras, Patras, Greece

Introduction

Archaeological evidence indicates that the bioprocess of food fermentation was discovered accidentally thousands of years ago, while up until today fermented foods display an important role in human nutrition (Adewumi, 2019). From the early days of civilization, fermented foods were produced by spontaneous fermentations having as main objective the preservation of raw materials. The consumption of fermented foods has been interwoven worldwide with disease prevention and the improvement of human health (Sõukand et al., 2015). The association between health-promoting effects and fermented foods was introduced by the scientific community in the mid-1990s when a Russian scientist, Élie Metchnikoff suggested that the consumption of milk fermented with lactobacilli could prolong the life of consumers (Mackowiak, 2013). Metchnikoff observation lighted the torch for exploitation of the health-promoting potentials of lactic acid bacteria and their fermented food products. As a result, food fermentation received increasing attention, highlighting by evidence-based investigations that live microbial cultures can provide health beneficial effects to the consumers (Adewumi, 2019; Macori & Cotter, 2018; Marco et al., 2017; Nampoothiri et al., 2017; Sõukand et al., 2015) According to many recent studies, consumption of fermented foods containing living microbial cultures could provide a plethora of different health benefits such as production of antimicrobial compounds against potential pathogenic microorganisms, anti-mutagenic, anti-carcinogenic, anti-tumor effects, amelioration of metabolic and physiological disorders, cholesterol reduction, enhancement of probiotics, production of exopolysaccharides (EPSs) which can be used as prebiotic ingredients and production of various other bioactive compounds which can positively affect the consumer (Adewumi, 2019; Chen et al., 2017; Marco et al., 2017; Singh et al., 2018).

The term "fermentation" is nowadays being used as the transformative effect of live microbial cultures and their metabolites on foods (Sõukand et al., 2015). Many different substrates such as milk, fruit and vegetables, meat and seafoods, cereal grains, and various miscellaneous food commodities have been used for ages in different parts of the world as a substrate for food fermentation (Ly et al., 2018; Marco et al., 2017; Misihairabgwi & Cheikhyoussef, 2017). Most of these food substrates have been proved to be easily affected by spoilage and pathogenic microorganisms in their raw states resulting in the foods' short shelf-life (Petruzzi et al., 2017). On the other hand, fermentation, which is a natural process, has been proved to extend shelf-life and safety of food products from the early days of civilization. According to legends, a farmer tried to store raw milk in the stomach of a cow for several days during summer, and when he checked his product after a few days, he was surprised to find out that milk had turned into a fermented white beverage of sour taste. The fermentation of milk came as a result of high summer temperatures and microflora occurring within the dried stomach of the cow. Likewise, many other food substrates could be fermented by naturally occurring microflora manufacturing a great variety of fermented foods. The production of traditional fermented food can be achieved through spontaneous or back-slopped fermentation. Back-slopping has been widely used by home-made or small-scale producers and is a process where a small sample of about 2–5% from a previous successful fermented food is incorporated into raw substrate to facilitate the initiation of a new fermentation (Ly et al., 2018). Subsequently, both cases (spontaneous or back-slopped fermentation) result in a strong naturally adapted starter culture. These autochthonous microbial communities are believed to derive from the raw food microflora, fermentation vessels, processors, other ripening environment equipment, or contaminants and dominate each substrate through competitive adaptation and favored environmental conditions (Bassi et al., 2015; Lorenzo et al., 2017; Ogunremi et al., 2017). Moreover, foods fermented by natural inoculation of autochthonous microbial strains can promote food safety and post-harvest preservation in parallel with enhancing nutrients' bioavailability, products' functionality and development of attractive organoleptic characteristics (Chen et al., 2017).

Regulation and Historical Evidence of Fermented Greek Foods

Fermentation has been reported to occur from old centuries of tradition in Greece while it was first documented by Homers during the 8th century BC. Homers describes the shepherd and cheese maker Polyphemus who got introduced during his journey to Odyssey, clearly providing documentation about milk fermentation. Likewise, in Odyssey is also introduced a mostly likely fermented type of sausage described as smoked goat-paunches filled with blood and fat. Likewise, traditional Greek sausages are nowadays being produced with lean meat and fat and the addition of salt, sugar, and various preservatives (phosphates, nitrites, monosodium, ascorbic acid) while various seasonings and herbs may be added (Gounadaki et al., 2007). Regarding fermented milk products, the climate and terrain of Greece has tended to favor the breeding of goats and sheep over cattle and thus many traditional cheese products are being produced by their milk including Feta, Batzos, Kalathaki of Limnos, Sfela, Anevato, Galotyri, Katiki, Kasseri, Graviera, and Kefalotyri (Litopoulou-Tzanetaki & Tzanetakis, 2011). Traditional Greek yogurt as well as kefir and sour milk also long history of production among Greek fermented products and they are classified according to the starter culture used during milk fermentation (Litopoulou-Tzanetaki & Tzanetakis, 2014; Megalemou et al., 2017). Finally, Greek table olives (natural black) occurring from Greek olive trees have a long history of production and they are widely known for their high nutritional value (Alexandraki et al., 2014). Most of these traditional Greek products are being included as basic components of the Mediterranean diet while according to European regulations they are characterized by specific designations of origin (Serra-Majem et al., 2019).

The European Union (EU) has applied specific regulation as part of food quality policy with respect to traditional foods. Three types of quality labels have been administrated regarding agricultural products and foodstuffs: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG) (European Commission, 2013). PDO is considered as the most important regulation which defines agricultural products or foodstuffs that are produced, processed and prepared in a specific geographical area while their quality characteristics are essential or exclusive due to the particular geographical environment and give specific importance to the "natural and human factors".

Types of Traditional Greek Foods

Fermented Dairy Products

Historically, the fermentation process of milk involved unpredictable slow souring of milk caused by the indigenously occurring microorganisms. Nowadays, traditional dairy products are being produced by modern microbiological processes preserving in parallel the high-quality nutrients initially occurring in fresh milk (Khedkar et al., 2016). According to the Codex Standard, fermented milk can be classified as fermented milk, concentrated fermented milk, and flavored fermented milk while all products must have a minimum content of 40% fermented milk (Codex Stan 243–2003). In general, fermented milk can be defined as a milk product prepared by specific microbial cultures. Fermented milk represents a great variety of products in different parts of the world, and each region provides different names usually identified by the use of starter cultures or labeled microorganisms, fat or protein content or manufacturing procedures (Khedkar et al., 2016). The numerous types of fermented milks produced around the globe include yogurt, acidophilus milk, koumiss, kefir, sour milk, leben, cultured butter milk, nunu, and ylette (Gonfa et al., 2001; Koutinas, 2017; Owusu-Kwarteng et al., 2017; Terpou et al., 2017) while they can be produced by different kinds of milk substrates such as ovine, caprine, cow, buffalo, donkey or even camel (Aspri et al., 2018; Ranadheera et al., 2018; Shori, 2017). In general, fermented milk products can be classified into three main categories: (i) sour to very sour types, e.g. yogurt; (ii) sour types with pleasant aroma, e.g. cultured milk; and (iii) acid-alcoholic, e.g. kefir.

Yoghurt

Yogurt is a fermented dairy product consumed worldwide, and due to its recognized health benefits, yogurt consumption has increased in recent decades. Greek yogurt is a fermented milk product and can be produced by different kinds of milk such as cow, ewe or goat and has been traditionally prepared by allowing milk to sour at 40–45 °C. In general, yoghurt production is achieved by milk fermentation through the synergistic action of lactic acid bacteria (LAB) such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* while various "wild" lactobacilli strains may occur depending on the origin of the starter culture (Terpou et al., 2017). During milk fermentation, the synergistic effect of the two microorganisms is highlighted. *S. thermophilus* grows faster by utilizing essential amino acids produced by *L. bulgaricus.* Due to the production of lactic acid by all LAB incorporated in the fermentation bioprocess, the pH of milk is reduced to 4.2–4.4 which is the optimal level for growth of lactobacilli. As a result, streptococci are inhibited whereas lactobacilli stay viable even at lower pH values in the range of 3.5–3.8. Greek yogurt acquires its unique character from bacterial metabolites and also by the use of nonstandardized or homogenized milk. This unique technique was maintained regarding traditional home-made yogurt production. Farmers used to produce yogurt by slight heating of raw milk up to 80 °C and proceeding with back-slopped fermentation. The temperature was maintained around 40–45 °C by covering the milk with a blanket and placing it close to the fire. As a result, fermentation of unstandardized milk was initiated by a previously used lactic acid culture while autochthonous microflora could also occur. The home-made fermentation provided a unique product with the formation of a crust on yogurt's surface (Megalemou et al., 2017). This crust was mostly liked by consumers, which turned the food industry to use the same traditional procedures in manufacturing yogurt.

Feta Cheese

In many milk-producing countries, a large fraction of the produced milk is used for cheese making. Likewise, the countries that consume cheeses the most are France, with 26.3 kg/capita; Germany, with 24.2 kg/capita; and Greece, with 23.4 kg/capita (Licitra et al., 2019). Numerous varieties traditional cheeses are produced throughout Greece nowadays. In general, Greek cheeses can be classified according to their texture and manufacturing technology, such as brined cheeses, soft cheeses, semi-hard cheeses, hard cheeses, and whey cheeses. Feta is white, brined, semi-soft traditional Greek cheese of protected destination of origin (PDO) and is one of the most important exporting products of the country (Terpou et al., 2018). Feta is characterized by a mild rancidity and a slightly acidic, salty taste. It is actually a white cheese of smooth, firm, and sliceable texture and this is actually why it was so named “Feta” which in English means “slice” (Michaelidou et al., 2003). In Greece, the annual consumption of Feta cheese is estimated at 12 kg per capita (Moatsou & Govaris, 2011).

Feta was traditionally produced by sheep and goats’ raw milk and the addition of a bacterial culture by back-chopping or addition of yogurt. Nowadays, according to Greek regulations, Feta cheese can be manufactured by pasteurized and standardized sheep milk or a mixture of sheep and goats’ milk, of which the latter must not exceed 30% of the total cheese milk. In all cases, milk is checked for the presence of antibiotics before it is submitted for coagulation. Manufacture usually occurs in well-equipped cheese dairies in which milk fermentation is achieved by the addition of rennet enzyme and a commercial lactic acid culture as starter. Rennet is an exogenous enzyme used as a milk-coagulant during Feta cheese production targeting to produce high-quality cheeses of good texture and flavor. Traditionally, calf rennet was applied in cheese production while nowadays alternative sources are being used such as rennet extracted from microorganisms (Abada, 2019). Traditionally, rennet enzyme is added into milk, mixed well and allowed to rest for 30–50 min at 30–35 °C before the inoculation of the starter culture. Regarding starter cultures, the most well-established starter for Feta cheese production is the commercial yogurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Other commercial cheese culture methods used in Feta cheese production include *Lactobacillus lactic* subsp. *lactis* and *Lactobacillus lactis* subsp. *cremoris*. Subsequently, many recent studies have focused on the application of various probiotic strains as starter or adjunct cultures for Feta cheese production also followed in many cases by industrial applications (Angelopoulou et al., 2017; Papadopoulou et al., 2018; Terpou et al., 2018). Finally, according to Greek legislation standards, Feta cheese is obligated to mature for at least two months before it is ideal for consumption (Terpou et al., 2018). Subsequently, the selected starter culture (0.5–1.0%) and calcium chloride (10–20 g/100 kg of milk) are added for milk fermentation. If the starter culture occurs in a dried form, its activation is mandatory and is so achieved by suspending the dried culture into sterile milk for 10–15 min. The mixture is left for curd formation. When milk coagulation is achieved, the curd is cut in small cubes (1–2 cm wide) and left undistributed for 10 min. Subsequently, the curd is transferred into rectangular molds which are turned over at appropriate times during 18–24 h (16–18°C) targeting whey draining. The following day, the cheese is removed from the molds, cut into blocks, and dry salted around its surface. Cheese ripening is performed in two stages. During the 1st ripening period, cheese is placed temporarily in either wooden barrels targeting assimilation with traditional fermentation or metal containers, where dry salt is added between block layers. Then brine is added (up to 12% w/v NaCl) and cheese is maintained at room temperature (18–22 °C) for approximately 10–15 days until the pH drops to 4.6. During the 2nd ripening period, the old high salt concentrated brine is replaced by fresh brine (5% w/v NaCl) and cheese transported into cold storage (4 °C) for further ripening to achieve at least a total of 60 days (Terpou et al., 2018). The final Feta cheese must be free from possible spoilage or pathogenic microorganisms and the levels of moisture and fat-in-DM must be the maximum of 56% and minimum of 43%, respectively.

Kefir

Kefir is a fermented milk beverage consumed for thousands of years and is believed to originate from Caucasus, Tibetan or Mongolian mountains (Guzel-Seydim et al., 2011). The name kefir originates from the Slavic word “Keif” which means “living well” probably due to the overall sense of health and well-being provided by its consumption (Rosa et al., 2017). Kefir has been traditionally produced in Greece as a functional beverage with known health benefits even though not many studies exist regarding its regional microbial diversity (Kalamaki & Angelidis, 2017). As previously mentioned, kefir’s functional character owns its label being an excellent source of probiotics while one of its main features is that its grains may be recovered after fermentation. The microbiological composition of kefir grains is still controversial while it is basically believed that it is composed of a natural mixed culture in which lactic acid bacteria (LAB), yeasts, and other bacteria co-exist in symbiotic associations. Different reports indicate that kefir grain microflora strongly depend on the origin of the grains, on the local conditions of culture, and on the storage and elaboration processes (Dertli & Çon, 2017; Fiorda et al., 2017). According to the Codex Alimentarius (Codex Stan 243–2003), kefir is a complex fermented dairy product created through the symbiotic fermentation of milk by lactic acid bacteria and yeasts contained within an exopolysaccharide and protein complex called a kefir grain. Kefir starter culture can be prepared from kefir grains, *Lactobacillus kefiri*, and species of the genera *Leuconostoc*, *Lactococcus* and *Acetobacter* growing in a strong specific relationship (Likotrafiti et al., 2015). Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguus*). Likewise, the microbiota present in kefir and kefir grains include numerous bacterial species from lactic acid and acetic acid groups, yeasts and filamentous fungi, which develop complex symbiotic associations (Dertli & Çon, 2017). The microbial composition of kefir may also vary according to culture medium, origin of kefir grains, different production techniques, different cultivation temperatures, type and composition of fermentation medium, and storage conditions (Gaware et al., 2011). The total number of microorganisms in kefir produced by milk fermentation should be at least 10^7 CFU/ml of bacterial cell culture, while the yeast number should not be less than 10^4 CFU/ml (Codex Stan 243–2003).

Kefir is mainly characterized by a distinctive sour flavor which may vary depending upon its intensity from batch to batch. Typically, kefir is produced by the incubation of kefir grains in milk at room temperature (25 °C) for up to 24 h and a final pH of approximately 4.6 (Guzel-Seydim et al., 2011). Kefir grains are composed of microorganisms immobilized within a polysaccharide and protein matrix. This polysaccharide of kefir is called kefiran and comprises 50% of the mass (dry basis) of kefir grains. Kefiran is mainly colonized in the broader term of grains, and can subsequently affect the rheological characteristics of the final product (Guzel-Seydim et al., 2011). *Lactobacillus kefiran* is primarily responsible for the production of kefiran and it can be affected by fermentation temperature, pH and various solutes in aqueous solutions but not fermentation time (Exarhopoulos et al., 2018a). In addition, the production of kefiran is also reported by *Lactobacillus kefir*, *Streptococcus mutans*, *Leuconostoc mesenteroides*, and *Streptococcus cremoris* (Kalamaki & Angelidis, 2017). Kefiran can exert immunomodulatory, antimutagenic, antiulcer, antiallergic, and antitumor properties and can also act as prebiotic (Exarhopoulos et al., 2018b). Finally, inoculation of kefir grains in milk results in a unique slightly carbonated beverage which also contains small amounts of alcohol being characterized by a unique fizzy, acidic, tarred taste, and refreshing flavor (Dertli & Çon, 2017).

Traditional Fermented Meats

Greek Dry Fermented Sausages – Salami Aeros

Natural fermented sausages have a long history of production especially in the Southern European countries like Greece, Italy, and Spain (Rantsiou et al., 2006). Traditional Greek dry fermented sausage also known as “salami aeros” represents an important economical income of the Greek meat industry (Samelis et al., 1998). Dry fermented sausages are usually consumed raw while their unique aroma, which may vary according to the region or recipe of production, is related to the biochemical activities that occur during fermentation, maturation, and drying (Sidira et al., 2015).

Fermentation of meat has been used from the old times as a mechanism targeting to extend the shelf life of perishable raw meat products by naturally occurring microflora and the addition of various species and salt as preservatives. The manufacture of dry fermented sausages includes mixing of different varieties of minced or chopped meat with meat fat, salt, spices, and various other seasonings. The sausage mixture is stuffed in casings retrieved from the small intestine of pigs and then dried (cured) under controlled conditions. Drying usually takes place in cool rooms

(15–18 °C) with sufficient aeration. Dry fermented sausages are provided for consumption when the pH value of fermented meat is of 5.3 or less and the a_w is reduced to 0.90 or less (Pragalaki et al., 2013). Low pH values along with low moisture content postpone the action of microorganism and the sausages can be characterized as ready for consumption.

Traditionally dry fermented sausages are manufactured without addition of starter cultures. Fermentation is achieved by the synergistic act of "wild" strains mainly acquired from raw meat. Dry fermented sausages have been traditionally prepared in small processing units of local butchers or home-made. As a result, meat fermentation was achieved by autochthonous microflora while their population varied according to environmental conditions, variation of in-house flora and other process parameters. The main drawback of this procedure is that it is not possible to ensure that the population of microorganisms present in the raw meat would provide fermented products of the same quality avoiding the hazard of pathogens' accumulation (Roccato et al., 2017). On the other hand, industrially produced dried fermented sausages acquire the use of a starter culture in order to ensure the stability and safety of the product. Commercial starter culture may consist of pure or mixed strains of lactic acid bacteria, micrococcaceae, and yeasts and molds (Drosinos et al., 2007). As it has been well documented, the use of starter cultures in meat fermentation has various benefits as is the reduction of ripening time, improvement of products' sensory characteristics as well as inhibition of possible spoilage or pathogenic microorganisms by the production of acids and other antimicrobial agents (Rantsiou et al., 2006). Meat fermentation is a complex bioprocess accelerated by the desirable action of specific microflora. More specifically, in meat fermentation lactic acid bacteria possess the main role as they are most wanted in order to reinsure the products' microbial stability by the production of lactic and acetic acid and the consequent decrease of pH value. Micrococcaceae can reduce spoilage, decrease fermentation time and contribute to the color and flavor development. Finally, yeasts and molds have a significant part in the organoleptic sector of fermented sausages as they accumulate in carbohydrate fermentation, proteolysis, lipolysis, lactate oxidation, and degradation of amino acids (Drosinos et al., 2007). Various commercial starter cultures may lack strain variety and as a result the superior quality of fermented meat products attributed to the specific composition and metabolic activity of the indigenous microflora cannot be provided by these starters. On the other hand, the incorporation of "house flora" by back-chopping or by isolation of selected strains mixed with industrial starters may result in the production of traditional dry fermented sausages of superior characteristics (Papamanoli et al., 2003; Samelis et al., 1994).

Fermented Fruits and Vegetables

Table Olives

Table olives are currently the most important fermented vegetable of the food industry consumed worldwide (Doulgeraki et al., 2013). Fermented table olives are widespread in the Mediterranean region being one of the main components of nutrition. According to the International Olive Oil Council, it is estimated that Mediterranean countries and specifically Greece, Spain, and Italy supply the world market by approximately 30% of the annual production of table olives (IOC, 2018). Greece has recently been reported as the second largest exporter of table olives in the European Union while it is estimated that the consumption of table olives in Greece is about 1.8 kg per capita (Angela et al., 2015).

Greek fermented olives are traditionally produced by spontaneous fermentation of lactic acid bacteria encountered on natural black olives mainly from cv. Conservolea or Kalamata (Bleve et al., 2015). The table olive industry is up until nowdays highly traditional, and thus the fermentation of table olives is carried out in a brined environment by the incorporation of autochthonous microflora in both home-made and large-scale industrial production. Natural black olives are inedible after harvesting as oleuropein, a phenolic compound with a bitter taste, occurs within very high levels. In order to reduce oleuropein content and consequently lose bitterness, olives are directly brined after harvesting at a mature stage and consequently submitted to fermentation for 8–12 months (De Angelis et al., 2015). During fermentation, olive sugars are accumulated by the autochthonous microflora of natural black olives producing lactic and acetic acid which is finally released into the brine. As a result, the pH of the brine is reduced which in combination with the high salt concentration can verify the microbial safety of the product. In addition, small amounts of ethanol and other volatile by-products are also released during fermentation into the brine providing unique sensory characteristics to the final product (Doulgeraki et al., 2013).

Greek fermented olives are known for their high nutritional value while their consumption has been associated with various health benefits linked with specific nutritional or bioactive compounds, such as their antioxidant capacity, and phenolics, unsaturated fatty acids and tocopherols content (Alexandraki et al., 2014; Boskou et al., 2006). In addition, the microbial composition of fermented table olives can also be associated with various health benefits. In general, the final microbial population of fermented olives comes as a result of the complex interactions between the indigenous microbiota naturally occurring on the olives and the equipment and environmental microbiota accumulated during processing (Panagou et al., 2003). As a result, the final microbial population of fermented table olives is mainly populated by lactic acid bacteria, yeasts, and, occasionally, molds. Interestingly, according to recent studies bacterial as well as yeast isolations from fermented table olives are possible candidates for probiotic delivery (Argyri et al., 2016; Bautista-Gallego et al., 2013; Oliveira et al., 2017). Thus, Greek fermented olives can reasonably claim a spot between functional food products.

Future Perspectives

Based on historical and traditional knowledge, a growing body of novel scientific reports has risen regarding traditional fermented foods and their functional characteristics. A plethora of impressive scientific and technological developments have resulted in raising attention towards microbial cultures and their use in traditional food fermentation. With increasing public interest in health, the effective health-related elements of the traditional fermented food have come under scrutiny. Scientific reports have documented that the benefits of fermented foods are likely greater than the sum of their individual bioactive or nutritional components. Therefore, it is of critical need to investigate in future studies the effects of different fermented foods and their associated bioactive compounds on human health. To do so, there should be applied novel scientific techniques as are metabolomics in conjunction with microbial ecology/genomics targeting identification of novel bioactive compounds associated with fermented foods which in combination with placebo trails can lead to a new era in food innovation.

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Section 3

Plant-Based Fermented Food Products

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8

Plants Used as Bread Yeast in the Balkans from an Ethnobotanical Point of View

Anely Nedelcheva
Faculty of Chemistry and Pharmacy, Sofia University 'St. Kliment Ohridski',
Sofia, Bulgaria

Yunus Dogan
Buca Faculty of Education, Dokuz Eylül University, Izmir, Turkey

1 Introduction

The striving of the modern man towards an overall well-being includes healthy food as an essential element. According to modern perceptions, food not only needs to satisfy the body's needs for energy and nutrients, but it also needs to have a preventive function. That is why nutrition should be healthy, balanced, and adequate to our energy needs. Traditional foods are one of the main features of the individual stages of the development of society. They are related to the health and longevity of different peoples. Today's recommendations go along the same lines—to return to traditional foods and not allow them to be replaced by the modern diet today.

The topicality of the issue 'traditional foods' has been reflected in the past two decades in the numerous scientific studies of various teams and institutions at a global level (Kuhnlein et al. 2013). Ethnobotany, through the scientific reconstruction of the relationship and the interaction between man and plant, reveals and describes patterns of use of wild and cultivated plants for food, and it establishes and explains the levels and dynamics of transfer of traditional plant knowledge.

In recent years, the Balkans have been in the focus of ethnobotanical research that reveals the traditional knowledge of the model of diet and use of the means of healing in the light of cultural diversity (Pieroni and Quave 2014), placing it in a comparative matrix as part of the traditional knowledge on the Balkans and Europe. Diet on the Balkan Peninsula is close to modern requirements for a healthy diet (Figure 8.1).

In recent years, the relationship between ethnobotany and modern technology has focused on the notion of ethnophytotechnology. Some authors (de la Parra and Quave 2017) present the concept of the relationship of the scientific study of traditional knowledge and an integrated platform of enhancing the yield and analysis of ethnobotanically derived drugs with the tools of biotechnology and reciprocally informing plant biotechnology techniques with ethnobotanical information. Similarly, such a direct link can also be made to the relationship between ethnobotanical knowledge and the development of biotechnological processes in food production.

Several publications have summarized the knowledge of food preparation using fermentation processes and some of them focus on sourdough bread and related products (Hammes and Gänzle 1998). Some recent studies aim to isolate the yeast strains that could be used effectively as baker's yeast and compared them with the commercial baker's yeast available in the market and to identify these strains which carried a possibility of being used as industrial baker's yeast (Tika et al. 2017).

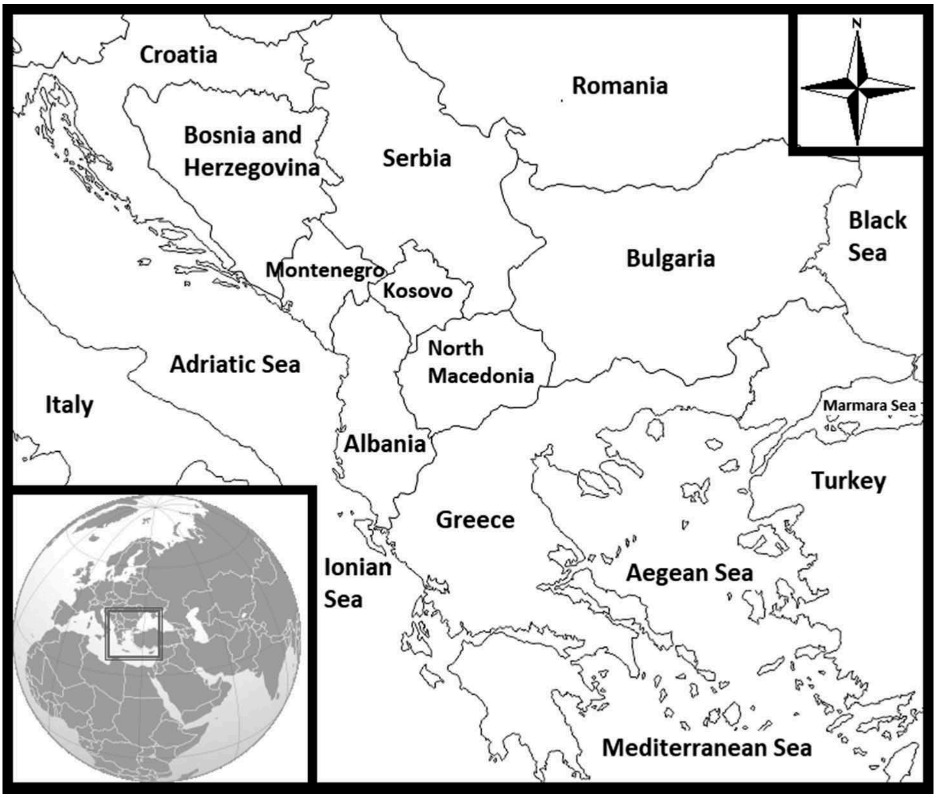


FIGURE 8.1 The map of the study area—the Balkans: historical and geographic area that includes countries on the Balkan Peninsula in the southeast of Europe.

2 Fermented Food

An important part of the food of people on the Balkans is fermented food and fermented beverages (soft and alcoholic). Fermented food includes bread, leavened pasta, dairy products, pickles, and fruit pickles. Fresh alimentary products turn sour (leavened) using leaven (sourdough). In the folk speech, the ferment for all these products are called in the same way Kvas = yeast, which shows a notion of the uniformity of origin and nature of the ferment. In the different languages of the population on the Balkans, there is a close sound and equal etymology—BG: мая, квас, квасец [maya, kvas, kvasets]; MK: квасец [kvasets]; RS: квасац [kvasats]; TR: maya; HR: kvasac; RO: drojdie de bere; AL: maja.

3 Bread as the Main Food

Bread is considered as the main food, and at the same time, it is much more than just ordinary food. Bread is seen as a symbol of prosperity and happiness. Often the word bread means food in general (Marinov 2003a, 2003b; Markova 2011; Pavlov 2001). Bread is of two kinds: unleavened and leavened bread. The emergence of leavened bread is associated with Egypt, and later it was brought to Greece, and in that early period, yeast was prepared from flour and grape strum. Later this practice spread throughout Europe.

The two types of bread are prepared and baked differently. In spite of the long history of making leavened bread, where all its components and processes are technologically streamlined, today there is a real revival of interest in its traditional production. Small-scale production of home-made leavened bread or such produced in bakeries is organized. There are several recipes for their preparation, as well as practical tips for successful results in modern conditions—using unprocessed flour (without improvers), spring water, and use of a glass or clay pot. Moreover, making handmade bread with leaven is the basis and symbol of some postmodern cultural communities in urban areas (Ek-Nilsson 2015).

4 Traditional Knowledge about the Leaven Preparation

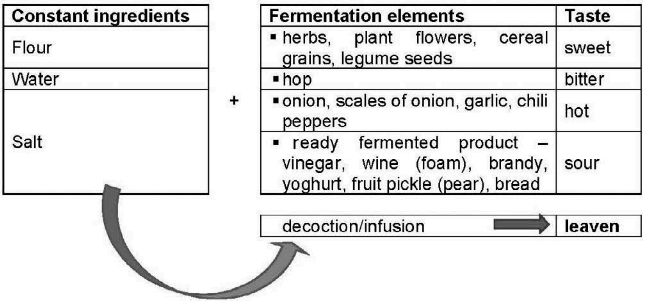
There are two ways to induce fermentation of the dough: (i) using 'old leaven' and (ii) preparing a new leaven called 'starting a new leaven' (for Bulgarians). In the preparation of leavened bread, constant dough ingredients + fermentation elements are involved (Figure 8.2).

Leaven is a homogeneous liquid mixture or smooth paste from decoction and a small amount of flour. For this purpose, the solid herbal ingredients are boiled—a decoction is prepared or soaked for a long time in hot water—as a result of which there is an infusion. Very common is the formula of the following decoction: hops/chickpeas + wheat/legumes + chilies + aromatic herbs (Figure 8.3). Knowledge related to making new bread yeast is regionally specific (Figure 8.4). A large number of home-made recipes have been found in documentary sources.

5 'Lost Leaven is Started Using a Plant'

This Bulgarian proverb shows that plants are considered an essential component of natural yeast.

As a result of the ethnobotanical studies in the Balkan region, more than 40 plant taxa have been established which are used in traditional practices as a fermentation element, over 30 of which are used in making bread yeast and 25 taxa of them have a high index of citation in documentary sources and by informants in field studies (Table 8.1). They are representatives of 24 families and 17 genera. The plants of the Poaceae (5), Leguminosae (3), Solanaceae (2) and Amaryllidaceae (*Allium* spp., 2) predominate. Dicotyledon plant taxa are dominant (72%). All taxa are well known to the local population as plant species used for food, spices, or medicinal plants. They are related to people's lifestyle, used in everyday life, easily accessible, and well recognizable. The number of wild and cultivated plants used for yeast is almost the same. This is yet another confirmation that cultural characteristics are seen as the leading reason for choosing the use of the plant at the expense of local flora and biogeographical features.



Constant ingredients
Flour
Water
Salt

+

Fermentation elements	Taste
▪ herbs, plant flowers, cereal grains, legume seeds	sweet
▪ hop	bitter
▪ onion, scales of onion, garlic, chili peppers	hot
▪ ready fermented product – vinegar, wine (foam), brandy, yoghurt, fruit pickle (pear), bread	sour

decoction/infusion ➡	leaven

FIGURE 8.2 A general formula for preparing sourdough with natural products.

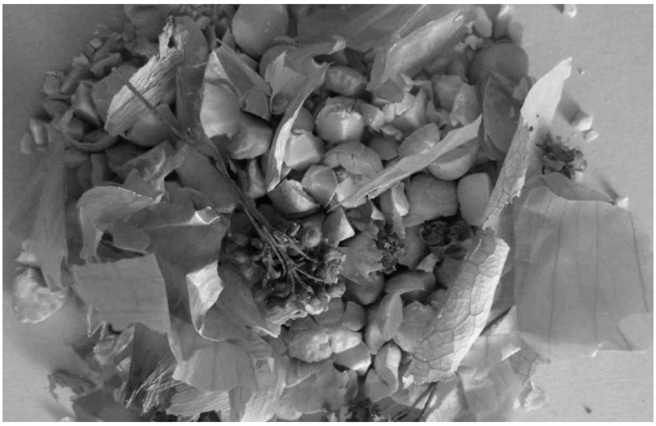


FIGURE 8.3 A herbal mixture for yeast starter from fragmented seeds of chickpea, flowers of the lime tree, blossoms of the common hop, and dry scales of the onion bulbs (Bulgaria).

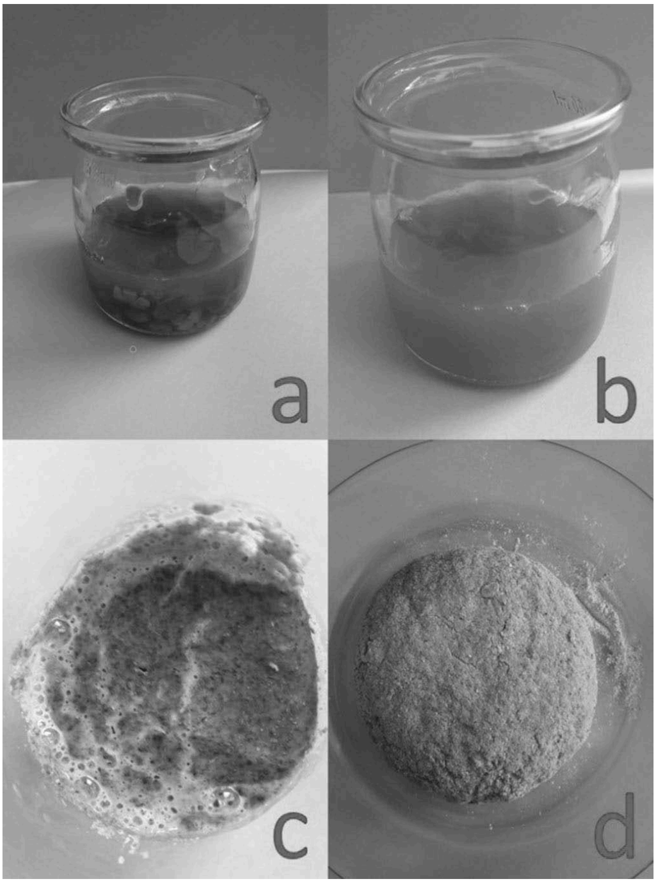


FIGURE 8.4 Method of making the traditional leavened bread in Bulgaria: herbal decoction (a), strained liquid for starter (b), sourdough starter—a culture of flour and herbal decoction (c), a dough ready for rising and baking (d).

TABLE 8.1

The list of the plants as bread yeast in the Balkan countries

Taxa and Family	Local name(s)	English name	Used part(s)	Country	Status	Source(s)
Allium cepa L.*, Amaryllidaceae	Luk (Лук)^{BG}	Onion	Bulb (scales)	Bulgaria	C	Markova (2011), Sõukand et al. (2015)
Allium sativum L., Amaryllidaceae	Chesan (Чесън)^{BG}	Garlic	Aerial parts, bulb (scales)	Bulgaria	C	Markova (2011)
Avena sativa L., Poaceae	Oves (Овес)^{BG}	Wild oats	Grain	Bulgaria	C	Markova (2011)
Beta vulgaris L., Amaranthaceae	Tsveklo (Цвекло)^{BG}	Beet	Root	Bulgaria	C	Sõukand et al. (2015)
Capsicum annuum L., Solanaceae	Lyuta chushka (Люта чушка)^{BG}	Hot pepper	Fruit	Bulgaria	C	Markova (2011)
Cicer arietinum L.*, Leguminosae	Leblebia^{YÖ}, Nohut^{TR}, Leblebiya (Леблебија)^{MK} Nahut (Нахут)^{BG}	Chickpea	Seed	Bulgaria, Macedonia, Turkey	C	Markova (2011), Say and Güzeler (2016), Nedelcheva et al. (2017)
Clematis vitalba L., Ranunculaceae	Kurpna^{MK}	Traveller's joy	Flowers	Macedonia	W	Pieroni et al. (2013)
Hordeum vulgare L., Poaceae	Echemik (Ечемик)^{BG}	Barley	Grain	Bulgaria	C	Markova (2011)
Humulus lupulus L., Cannabaceae	Şerbetci otu^{TR}, Mayaotu^{TR}, Hmel (Хмел)^{BG}	Common hops	Flower, leaf	Bulgaria, Turkey	W	Kültür (2008), Cansaran and Kaya (2010), Markova (2011)
Juglans regia L., Juglandaceae	Orev (Орев)^{MK}	Common walnut	Fruit, outer pericarp	Macedonia	C	Rexhepi et al. (2018)
Laurus nobilis L., Lauraceae	Dafinov list (Дафинов лист)^{BG}	Laurel	Leaf	Bulgaria	C	Markova (2011)
Malus sylvestris (L.) Mill., Rosaceae	Molla e egër^{AL}	Crab apple	Fresh unripe fruit	Albania	W	Pieroni and Sõukand (2017)
Ocimum basilicum L., Lamiaceae	Bosilek (Босилек)^{BG}	Basil	Aerial parts	Bulgaria	C	Markova (2011)
Phaseolus vulgaris L., Leguminosae	Fasul (Фасул)^{BG}	Common bean	Dried seed	Bulgaria	C	Markova (2011)
Pimenta dioica (L.) Merr., Myrtaceae	Bahar (Бахар)^{BG}	Pimenta	Dried unripe fruit	Bulgaria	C	Markova (2011)
Salix spp., Salicaceae	Varba (Върба)^{BG}	Willow	Flower	Bulgaria	W	Markova (2011)
Secale cereale L. Poaceae	Razh (Ръж)^{BG}	Rye	Grain	Bulgaria	C	Sõukand et al. (2015)

(*Continued*)

TABLE 8.1 (Cont.)

Taxa and Family	Local name(s)	English name	Used part(s)	Country	Status	Source(s)
Sempervivum spp., *S. macedonicum* Praeger, *S. hirtum* L., Crasullaceae	Debelets (Дебелец)^{BG}, Babin kvas (Бабин квас)^{BG}, Čuvarkuća žuta^{BiH}	Houseleek	Leaf (juice)	Bulgaria, Macedonia, Bosnia and Herzegovina	W	Markova (2011), Rexhepi et al. (2018)
Solanum tuberosum L., Solanaceae	Kartof (Картоф)^{BG}	Potato	Tuber (unpeeled)	Bulgaria, Macedonia	C	Markova (2011)
Tilia spp., Malvaceae	Lipa (Липа)^{BG}	Lime tree	Flower	Bulgaria	W/C	Markova (2011)
Triticum spp.*, Poaceae	Zhito (Жито)^{BG}	Wheat	Grain (green spike)	Bulgaria	C	Markova (2011), Sõukand et al. (2015)
Vaccinium myrtillus L., Ericaceae	Boronicë^{AL}	Bilbery	Fruit	Albania	W	Sõukand et al. (2015)
Vicia sativa L., Leguminosae	Burchak otu^{YÖ}	Common vetch	Aerial part	Macedonia	W	Nedelcheva et al. (2017)
Vitis vinifera L., Vitaceae	Rrush^{AL}	Grape	Fresh unripe fruit	Albania	C	Pieroni and Sõukand (2017)
Zea mays L., Poaceae	Tsarevitsa (Царевица)^{BG}	Maize	Grain	Bulgaria	C	Markova (2011)

* Additional to bread yeast, it is used as yogurt yeast too.

AL: Albanian, BG: Bulgarian, BiH: Bosnian, MK: Makedonian, TR: Turkish, YÖ: Yörük dialect (Turkish) in North Macedonia

C: Cultivated, W: Wild

The morphological parts used and the type of their primary processing varies. Typically, only one morphological part is used from each plant. And in some cases, the exact vegetative phase is given (unripe fruit, green spike), a detailed part of the plant (bulb scales, fruit, outer pericarp, leaf (juice) or a particular condition of the plant part (fresh, dry, unpeeled tuber).

The conditions for the development of the microorganisms necessary to obtain fermentation products are related to a complex of chemical compounds and organic matter of plant tissues which can be provided by a specific stage in the development of the plant and its parts. The empirical experience of people on the use of plants primarily for food and medical treatment has led to the selection of precisely defined objects as a fermentation element.

Fruits and seeds are the most commonly used parts. Fresh unripe apples and grapes are used for yeast (Figure 8.5), while ripe fruit is used for food. These unripe fruits have been used in the dietary pattern in the past as a food acid for sourness (Nedelcheva 2013), and are therefore also part of the diet. The pericarp of unripe walnuts is widely used as a medical and dye plant (Dogan et al. 2003). Seeds of chickpeas and beans are part of the main protein vegetable foods. Main cereals are also a major part of the established species—wheat, corn, oats, barley, and rye. Unripe green ears of wheat, which is the most popular grain flour to start leaven, are used. All of them are a constant ingredient of bread (flour) and a source of leaven at the same time. They are the link between the mandatory ingredient and the fermentation element of bread. Garlic, onion, and chilli peppers are an integral part of the region's culinary characteristics (Figure 8.6). Bay leaf and pimento are foreign species but well integrated in local traditions as spices (Figure 8.7). They are essential oil plants like basil (a medicinal and ritual plant) (Marinov 2003a, 2003b; Nedelcheva and Draganov 2014).

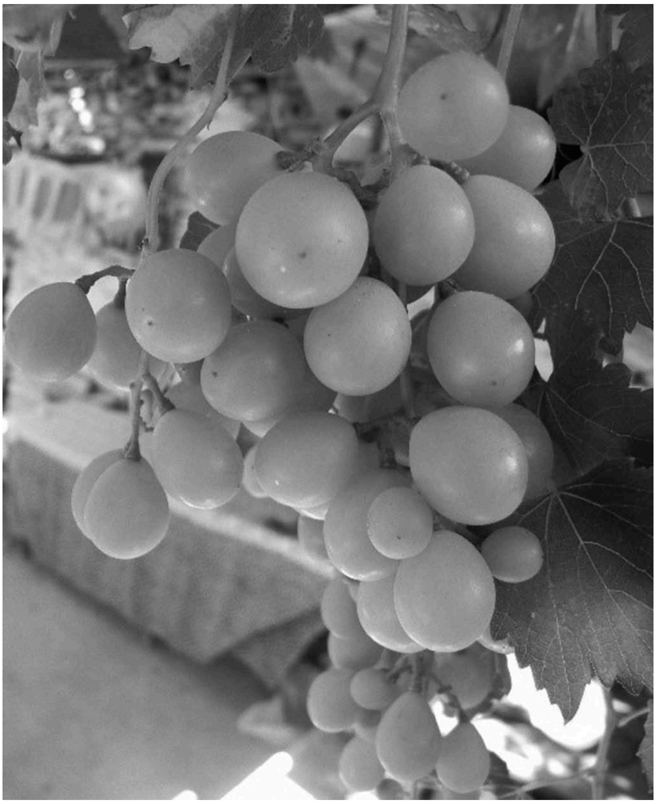


FIGURE 8.5 Unripe fruits of *Vitis vinifera* for bread yeast in Albania.

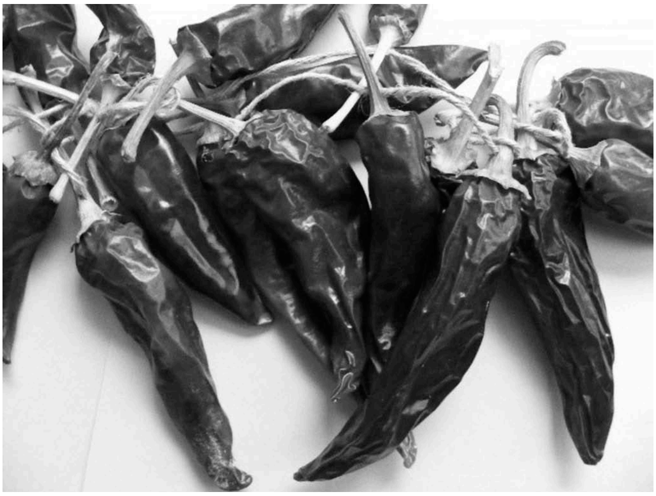


FIGURE 8.6 Hang-dried hot peppers (*Capsicum annuum*) in Bulgaria.

FIGURE 8.7 Dried leaves of *Laurus nobilis*.

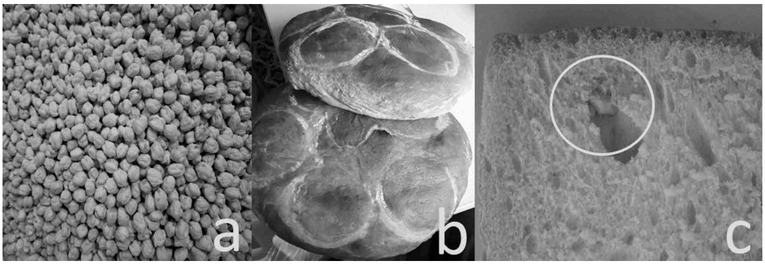


FIGURE 8.8 Chickpea (a) used as a starter for dough; traditionally consumed chickpea bread in Beydağ (Izmir, Turkey) (b), and a piece of chickpea inside bread (c).

Lime flowers are one of the most widely used herbal teas that are consumed as a refreshment drink. An interesting find is the use of the willow racemes. Here an analogy can be made with the use of male hazel racemes (*Corylus avellana* L.) to make flour (in a period of famine) (Nedelcheva 2013).

Which of these plants can be identified as cultural markers in connection with the preparation of bread yeast on the Balkans? *Cicer arietinum*, chickpeas is of primary importance as a fermenting element for the area (Figure 8.8), which is also used today and is of interest to science. Studies of chickpea steep liquor (CSL) as a dough-leavening agent show that CSL could be used as an alternative to yeast for syngas fermentation. On the other hand, CLS is rich in nutrients and lower in cost compared to yeast (Saad et al. 2015).

Hops is also widely used, and its scientific name is *Humulus lupulus*. The hop extract is used as an antifungal ingredient for bread making and selection of autochthonous-resistant starters for sourdough fermentation (Nionelli et al. 2018). The use of beet as a source for starter cultures for different fermented foods (cheese, meat, sourdough, etc.) is well known and has been applied by Asian and Mediterranean people for the past 4000 years (Hui and Evranuz 2012). The isolation of yeast from *Capsicum annum* is reported as well as its probiotic potential (Di Cagno et al. 2009, Lara-Hidalgo et al. 2019). Legumes, including dry been, are well known as sourdough starters. The sourdough fermented legume flours are suggested for the manufacture of novel functional foods and/or pharmaceuticals preparations (Rizzello

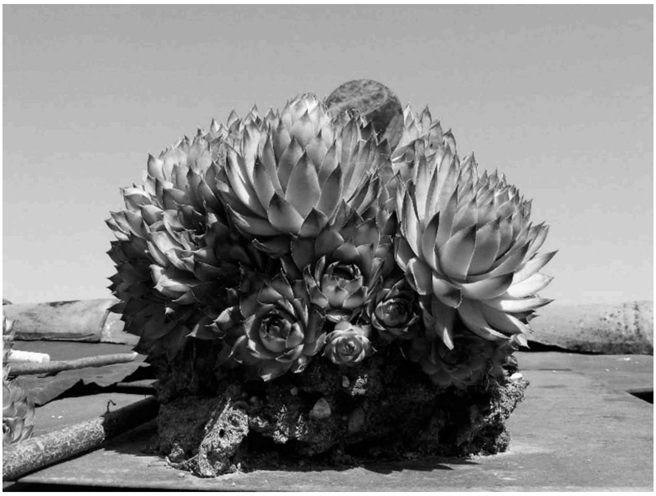


FIGURE 8.9 *Sempervivum* leaves used for bread yeast in North Macedonia.

et al. 2015). Potatoes and starch are also well known for such use, but today, they are also the subject of research on the sensory properties of wheat bread (Pavilonytė et al. 2015). Contemporary research on dough fermentation and sensory, structural, nutritional, and shelf life properties of leavened baked products is associated with some of the established species. Isolated *yeast strains (Saccharomyces cerevisiae)* from grapes show a strong potential for commercialization after appropriate research on biomass optimization culture preservation (Tika et al. 2017).

Interesting is the reported use of houseleek as a leavening agent. *Sempervivum* spp. is a well-known plant whose leaf juice is used for the treatment of ear diseases and has a pronounced antibacterial effect (Stojičević et al. 2008). It is also established as a cultural marker for some small communities on the Balkans with its healing properties (Nedelcheva et al. 2017) (Figure 8.9). Its use as a fermentative element is according to ethnobotanical data of its past use and its Bulgarian common name 'babin kvas' (grandma's sourdough).

According to the available data in scientific ethnobotanical papers, there are also some new reports on the use of willow racemes (*Salix* spp.), lime flower (*Tilia* spp.), bilberry fruit (*Vaccinium myrtillus*) and the use of bay leaf and pimento as ingredients of leaven (Figure 8.10).

Species not found in Europe and the Balkans that are used to start leavening are also described in various papers: *Physalis peruviana* L. (Solanaceae) is used as yeast in India, and Compositae members *Inula cappa* (Buch.-Ham. ex D.Don) DC and *Acmella paniculata* (Wall. ex DC.) R.K.Jansen are used in Nepal (Bhattarai et al. 2011). In Nepal, the Asian species *Clematis montana* Buch.-Ham. ex DC. is used while on the Balkans (the area studied) *Clematis vitalba* is used as a sourdough starter.

Regardless of the concept of uniformity of the ferment, most of the plants are associated with the preparation of a specific product: bread or milk or cheese, except for the widely used onions, chickpeas, and wheat, which are used as yeast in bread and milk.

Several plants have folk names that indicate their use in starting yeast for different types of products, as with the Turks *H. lupulus* is 'Maya otu', *Gallium verum* L. is 'Yoğurt otu', and for cheese *Zosima absinthifolia* Link is 'Peynir otu'. The Yürüks call *Carlina acanthifolia* All. —'Maaya', due to its use in making cheese (Nedelcheva et al. 2017). The Bulgarians use the name 'babin kvas' (grandma's sourdough) to refer to houseleek *Sempervivum*. At the same time, none of the plants has a name that in all languages is directly related to its use as a basis for fermented food. This is another sign of the regional nature of this kind of traditional knowledge.

A fermented product may also be used as a fermentation element. Such is the fruit pickle (pear), which was a traditional food in the past for Bulgarians. In some regions a special type of

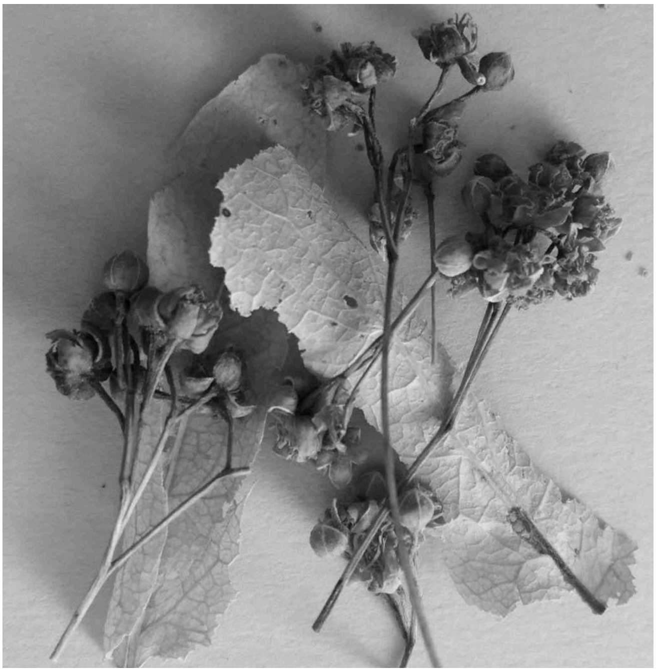


FIGURE 8.10 Dry flowers of *Tilia* used for bread yeast in Bulgaria.

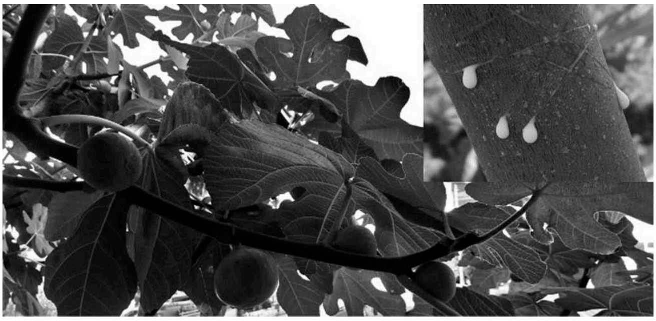


FIGURE 8.11 Latex of *Ficus carica* used for milk yeast.

fruit pickle was made from wild apples, pears, plums, blackberries (*Rubus caesius* L.), raspberries (*Rubus idaeus* L.), lingonberries (*Vaccinium vitis-idaea* L.), and medlars (*Mespilus germanica* L.) only or in combination (Markova 2011; Nedelcheva 2013; Sõukand et al. 2015).

In addition to the plants found in the area, such used for milk–*Rubus idaeus* (Rexhepi et al. 2018), fig (*Ficus carica* L.) and tournefort's gundelia (*Gundelia tournefortii* L.) are also said to be used as bread yeast (Say and Güzeler 2016) (Figure 8.11). The use of latex from Euphorbiaceae species for different products is interesting—latex from spotted spurge (*Euphorbia maculata* L.) for milk (Say and Güzeler 2016) and latex from Cypress spurge (*Euphorbia cyparissias* L.) for cheese (Nedelcheva et al. 2017).

6 Beliefs and Customs

In the past, leaven was an important element in every household. Sourdough was carefully stored in a special container intended only for this (usually a clay pot). According to beliefs, sourdough is not given or borrowed after sunset, when someone in the house has chickenpox, or when there is a young bride or a newborn at home (Georgiev 1999). The dried leaven was also worn as an amulet. A woman wearing leaven attracts her husband to grow fond of her and to love her, and a maiden who wears it turns into a flame for young men who are mad about her. Such a maiden gets married soon and moreover to the one she wants (Marinov 2003a, 2003b). Magical power is attributed to leaven. Leaven is a part of several rituals for the fertility of the fields, milk-yield of cows, preservation of the property and health of people (Georgiev 1999).

Starting new leaven for Bulgarians takes place on a certain day—Maundy Thursday. Maundy Thursday is the Christian holy day falling on the Thursday before Easter. It is full of symbols, and many rituals take place on this day. On this day, the leaven is renewed for the whole year—it is called new or young leaven. Dough for ritual bread is made using young leaven. The young leaven is kept during the year, and it is used as a cure and against magic, too (Marinov 2003a, 2003b). Similar customs are observed in many of the Balkan peoples; this is related to the common Slavic traditions (Radenković 2014).

7 Leaven as a Cure

The external application of leaven as fomentation for pneumonia, as a cure for wounds as a result of freezing or burning, and for acute finger or fingernail inflammation (a mixture of leaven, sol ammoniac and soap is used) is well known (Georgiev 1999). Nowadays leaven is used as a fomentation for headache, bruises, and boils, where the place is first massaged with olive oil. For headache after poisoning (for example with alkaloids), a fomentation can be prepared in the form of a headcover filled with fresh leaven, often mixed with other herbs for a stronger effect such as horse chestnut, horseradish or raw potatoes (Dimkov 2001; Douno 2012).

8 Conclusion

Ethnobotanical studies show a huge regional variety of plants used to start leaven with strict microregional specificity. Plants which are not part of natural flora but are related to cultural traditions and established dietary patterns are used. Analyzing traditional knowledge and reconstructing it with scientific methods is a source of information for new, natural, plant-based fermentation elements. Ever-increasing interest in functional foods and the use of products from fermentation processes such as prebiotics, which have numerous beneficial effects on the body, makes the gathering, studying and sharing this information extremely timely and responsive to preserving and effectively applying today's traditional knowledge. This is just another look back to the future.

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9

Fermented Soybean Foods in Asia

Khongsak Srikaeo
Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Muang Phitsanulok, Thailand

1 Introduction

Soybeans are a crucial crop in East Asia. It is the most acknowledged source of plant protein, which also contributes to a wide range of health benefits. The presence of biologically active proteins in soy has led to soy-derived foods being regarded as functional foods (1). Though a legal definition of functional foods is still lacking, they are claimed to have beneficial physiological effects in the body (2). Soy foods are allowed to make a heart beneficial health claim if they contain a mandatory 6.26 g of soy protein per serving (3).

Apart from proteins, soybean contains basic nutritive constituents, such as lipids, vitamins, minerals, and free sugar and it also contains isoflavones, flavanoids, saponins, and peptides that are of therapeutic value (4,5). Soy isoflavones are a class of polyphenols of vegetal origin with structure and chemical properties similar to those of 17-β-estradiol, which also have oestrogenic activity (6). In addition to hormonal effects, isoflavones may have other physiological effects, such as inhibition of tyrosine kinases, activation of natural killer cells, antioxidant activity, etc. (7). High isoflavone intake by Asian populations as compared to that of Caucasian populations has been epidemiologically associated to a better intestinal health and lower rates of cardiovascular and cancer diseases (8,9). These claims have contributed to a progressive increase of soy and soy-derived foods consumption worldwide. However, the unpleasant (or unfamiliar) odor of soy products has limited wide acceptance of soy milk and traditional soy-derived foods by the Western population (10). This has favoured the development of soy-derived beverages and soy-fermented foods containing additives to counteract the strong flavours of soy (11,12).

Soybean is consumed in two forms, unfermented (roasted and fried soybeans, soy powder, soy butter, soybean oil, etc.) and fermented (soy sauce, cheese, pickle, yogurt, etc.). Fermentation has been used since ancient times and the basic idea behind the fermentation process was to preserve the perishable food materials specially where there was scarcity of such foods. In the present scenario, fermentation is applied to improve bioactive components responsible for health benefits (13,14) and reduction of antinutritional factors (15,16).

Fermentation is the method used commonly to produce various soy products. It is been proven that fermentation can improve the physicochemical and sensory quality of the soy products. Soybean is fermented using bacteria (*Bacillus subtilis* and lactic acid bacteria) and fungi (*Mucor* spp., *Aspergillus* spp. and *Rhizopus* spp.) (17). Although various microorganisms are involved in the fermentation of different types of foods, the predominantly used strains are *B. subtillis* and *Aspergillus.* Health promoting actions of commonly used fermented foods across the world is the prevention of cardiovascular disease, cancer, gastrointestinal disorders, and some allergic conditions (18).

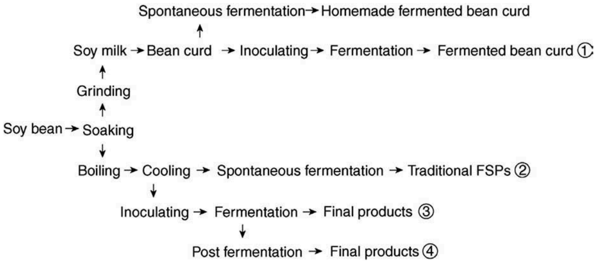
Spontaneous fermentation → Homemade fermented bean curd
Soy milk → Bean curd → Inoculating → Fermentation → Fermented bean curd ①
Grinding
Soy bean → Soaking
Boiling → Cooling → Spontaneous fermentation → Traditional FSPs ②
Inoculating → Fermentation → Final products ③
Post fermentation → Final products ④

FIGURE 9.1 Process flow chart and product classification of fermented soybean products. (1) Mold-fermented, bacteria-fermented, and enzymatically ripened bean curd. (2) Many species, mainly made at home now. (3) Tempeh, by *Rhizopus* spp., and natto by *Bacillus* spp. (4) Soy sauce, fermented black soybean, and fermented soybean paste.

Source: Reproduced with permission of Elsevier (20).

Fermented soybean products are very popular in many Asian countries such as Korea, China, Japan, Indonesia, Malaysia, Thailand, and Vietnam. Various types of fermented soybean foods are available. The popular soybean products include natto, miso, tofuyo (Japan), douchi, sufu, doubanjiang (China), chungkukjang, doenjang, kanjang, meju (Korea), tempeh (Indonesia), thua-nao (Thialand), kinema, hawaijar, and tungrymbai (India) (17). The most common fermented soybean foods of Korea are chungkukjang, doenjang, kochujang, and soy sauce. Natto and miso are Japanese versions of chungkukjang and doenjang, respectively. China also has various fermented soybean products such as doubanjiang, douchi, sweet noodle sauce, tauchu, yellow soybean paste, and dajiang (19). Although the raw material of all fermented soybean products is the soybean, their fermentation processes and relative microorganisms are very complex. The general process flow chart of popular fermented soybean products is shown in Figure 9.1.

This chapter provides information about the fermented soybean foods available in Asian countries. The production of fermented soybean foods including their health benefits and safety concerns is discussed.

2 Fermented Soybean Products

Fermentation of soybean gives rise to different products based on many criteria, but microbes are the foremost cause for the differences as they affect the aroma, texture, and therapeutical and neutraceutical values. *Bacillus* spp., lactic acid bacteria and fungi are reported to be the key players in fermented soybean products (21-24). Soybean products can be fermented solely by either *Bacillus* or fungi, but in some cases both are involved (17). Salt may be used during salted-type fermentation. Since salt prevents bacterial growth, fungi play a major role in the fermentation of salted soybeans. On the other hand, *Bacillus* is considered to contribute significantly to the fermentation of non-salted soybeans (25). Table 9.1 summarizes common fermented soy products, their origin, and key microbes.

2.1 Bacterial Fermented Soybean Products

Soybean fermented with *Bacillus* spp. results in alkaline fermentation, whereas soymilk fermented using lactic acid bacteria results in acidic fermentation. *Bacillus subtilis*-fermented soybean products are consumed in various countries and vary based on their traditional methods of preparation. Natto is a traditional Japanese food prepared by boiling soybeans and fermenting them with the *B. subtilis* natto. The extracellular enzymes from the bacteria act on the soybeans to produce mucilage, which gives the thread-like and slimy nature to natto. Natto has also shown to be a potential source of fibrinolytic enzymes and several bioactive peptides (23,26). Chungkookjang,

TABLE 9.1

Fermented soy products, their origin, and key microbes

Origin	Fermented soy products	Key microbes
China	Sufu	*Bacillus cereus*, lactic acid bacteria
	Fried yellow soybean sauce	*Bacillus subtilis*
	Stinky tofu	*Lactobacillus curieae*
	Yellow soybean paste	*Bacillus subtilis*
	Touchi (fermented black bean)	*Aspergillus oryzae*
	Fermented soy milk	Bifidobacteria
China and Taiwan	Dou-chi	*Aspergillus oryzae*
China and Japan	Natto (nattokinase)	*Bacillus subtilis*
Japan	Miso	*Tetragenococcus halophilus*
	Brown rice Koji	*Bacillus subtilis*
	Koji powder	*Aspergillus oryzae*
Thailand	Thua-nao	*Bacillus subtilis*
Indonesia	Tempeh	Fibrinolytic microorganisms such as *Bacillus subtilis*, *Staphylococcus sciuri*, *Enterococcus faecalis*, and *Citrobacter or Enterobacter*
India, Nepal and Bhutan	Knema	*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus thuringiensis* and *Bacillus sphaericus*
Korea	Chungkukjang	*Bacillus cereus*, *Bacillus amyloliquefaciens* and *Bacillus subtilis*
	Doenjang	*Bacillus subtilis* *Staphylococcus* *Sciuri* *Enterococcus faecalis*, *Citrobacter or Enterobacter*
	Kanjang	Total aerobic bacteria, lactic acid bacteria
	Kochujang	*Aspergillus oryzae*

a Korean traditional fermented soybean is also prepared by fermenting cooked soybean with *B. subtilis* as the starter culture (24). Chungkookjang exhibits various health benefits such as possessing antihypertensive (27), antidiabetic (28), and anticancer properties (29).

Fermented soybean varieties such as kinema, hawaijar, tungrymbai, and bekang are prepared traditionally and consumed in different states of India. Kinema has been reported to possess high antioxidant property due to increase in polyphenols and peptides during fermentation (30,31). Thua-nao is also an alkaline *B. subtilis* fermented soybean, which is very popular in the northern part of Thailand (32). Apart from *Bacillus* starter, soymilk fermented using different lactic acid bacteria is also a source of bioactive peptides and free isoflavones (33). Soymilk fermented with *Enterococcus faecium* strains have shown to possess antioxidant and angiotensin I converting enzyme inhibitory properties (34). Soy milk has been fermented using lactic acid bacteria such as *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium infantis*, *Bifidobacteriumlongum* (35), *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, and *Bifidobacterium breve* (36). Soymilk fermented with specific lactic acid bacteria results in peptides having specific health benefits (17).

2.2 Fungi-Fermented Soybean Products

Apart from bacterial fermentation, filamentous fungi are also used in several fermented soybean products in Asia to provide aroma, color, and biological activity. The popular fungi-fermented soybean products include douchi, tempeh, and miso tofu. Miso is a soybean paste prepared with soybean, rice or barley, salt and water with fungus *Aspergillus oryzae*. Preparation of miso takes place in three steps: first is fermentation of soybeans with salt to koji, second is the addition of lactic acid bacteria and yeast to improve the flavor followed by fermentation at 30°C, which results in miso, and the third is pasteurization and packaging. Douchi is a Chinese traditional fermented soybean product used for seasoning and for pharmaceutical purpose (37). *Mucor* and *Aspergillus* strains are associated with douchi fermentation, which involves a two-step fermentation process, which are primary and secondary fermentation (37,38). Douchi has been shown to possess antioxidant, angiotensin I converting enzyme, and α-glucosidase inhibitory properties (37,39,40). Tempeh is an Indonesian soybean fermented food produced by filamentous fungi, such as *Rhizopus* spp. and *Fusarium* spp (41). Tempeh exhibits a wide range of health beneficiary properties such as antioxidant, antimicrobial, anticancer, antihypertensive, antithrombotic, and hypocholesterolaemic effect (23,42,43). Tofu is a fermented soybean product which is widely consumed in East and Southeast Asia. In China and Taiwan, it is known as sufu, funan, tou-yu, tau-zu, fuyu, toufu-ru, rufu, toufu-ju, and furu, in Japan as tofuyo, in Philippines as tafuri, in Thailand as tau-fuyee, in Malaysia as tau ju, and in Vietnam as chao and dau-phu-nyu (44,45). In commercial practice, these fermented soybean products are prepared using molds such as *Actinomucor* spp., *Mucor* spp. and *Rhizopus* spp (44). Tofuyo has been reported to show antihypertensive and cholesterol lowering properties (46).

2.3 Combined Fungal–Bacterial Fermented Soybean Products

There are some fermented soybean products, in which both bacteria and fungi are involved in the development of the final product. Doenjang-meju is a Korean traditional fermented soybean paste, fermented using both bacteria and fungi. The bacteria involved in this process are *B. subtilis* and molds including *Rhizopus* spp., *Mucor* spp., *Geotrichum* spp., and *Aspergillus* spp. (47). *Bacillus subtilis* CSY191 isolated from doenjang was found to produce anticancer surfactin (29). It has also been reported that fermentation of soybean using both *Rhizopusoligosporus* and *B. subtilis* achieved higher degree of protein hydrolysis in comparison to a single starter (48).

3 Changes in Nutritional Values of Soybeans during Fermentation

Soybean products have been designated as one of world's healthiest foods due to their high-quality protein as well as various health benefits. The protein content of soybean is 32–42%, depending on the variety and growth conditions, of which approximately 80% is composed of two storage globulins, 7S globulin (β-conglycinin) and 11S globulin (glycinin), having various functional and physicochemical properties (49). Soybean products are considered a good substitute for animal protein, and their nutritional value is almost equivalent to that of animal protein because soy proteins contain most of the essential amino acids for human nutrition. Soybean products have less saturated fat and various phytochemicals (50). In addition, the association of high-quality protein and phytochemicals, especially isoflavones, is unique among plant-based proteins because isoflavones are not widely distributed in plants other than legumes. Soybeans contain 0.1–5 mg total isoflavones per gram, primarily genistein, daidzein, and glycitein (51). These nonsteroidal compounds, commonly known as soy phytoestrogens, are naturally present as the β-glucosides genistin, daidzin, and glycitin, representing 50–55%, 40–45%, and 5–10% of the total isoflavone content, respectively. Total isoflavonoid contents are varied among different soy products (50-52). In addition to high-quality protein and isoflavones, soybeans contain high levels of unsaturated fatty acids, dietary fiber, and minerals (19).

The qualitative and quantitative compositions of soybean components are dramatically changed by physical and enzymatic processes during fermentation (18). Fermentation is an excellent processing method for improving nutritional and functional properties of soybeans due to the increased content of small bioactive compounds. The large protein, lipid, and carbohydrate molecules in raw soybean are broken down by enzymatic hydrolysis during fermentation to small molecules such as peptides, amino acids, fatty acids, and sugars, which are responsible for the unique sensory and functional properties of the final fermented products. Short-term fermented soy foods such as chungkookjang and tempeh, which are fermented with *B. subtilis* and *Rhizopus oligosporus*, respectively, for less than 72 hours have a much greater concentration of large molecules than do long-term fermented foods including doenjang and miso, which are fermented for more than 6 months with *Aspergilus* and *Bacillus* species from rice straw and koji, respectively (49,53,54).

The degradation of lipids and carbohydrates proceeds rapidly during the initial stage of fermentation. Dry matter declines continuously during fermentation time, 70–80% of which is due to decreases in crude lipid and carbohydrate, which are used as the major energy sources for the microorganisms. The remaining loss is largely due to fermentative hydrolysis of approximately 25% of the soy protein, thereby producing ammonia. Of this hydrolyzed protein, 65% remains in the fermented products as amino acids and peptides, 25% is assimilated into the mold biomass, and 10% is oxidized. After the initial stage of fermentation, however, soy proteins are rapidly degraded and only 9–17% of the crude protein remains at the end stage of long-term fermentation (19,55).

Among the various small metabolites derived from macromolecules, the changes in amino acid and peptide concentrations were especially prominent. Amino acids increased or remained almost constant with increased fermentation time, but glutamate, the richest amino acid in soybean, was obviously decreased by fermentation, suggesting that microorganisms might use it as a preferred nitrogen source (56). Soybean fermentation releases many small peptides by the hydrolysis of soybean proteins by microbial proteases, which exhibit therapeutic properties (Figure 9.2). Bioactive peptides in fermented soybean are either formed by hydrolysis of soybean protein or produced by the starter culture.

Isoflavones, which are mostly present as 6-o-malonylglucoside and β-glucoside conjugates and associated with proteins in soybean, are also broken down by heat treatment and fermentation (57). During preparation of fermented soy foods, 6-o-malonylglucosides, the most prevalent soybean isoflavones, are converted to 6-o-acetylglucosides or β-glucosides by heating, and β-glucosides are unconjugated by the action of β-glucosidases secreted by fermentation microorganisms (52). Most isoflavones are not enzymatically hydrolyzed during short-term fermentation, in contrast to long-term fermentation in which 6-o-malonylglucoside content declines with increasing fermentation time with concomitant increases in unconjugated aglycones (genistein and daizein). In chungkukjang (Korea), the aglycones are 21 times higher than in soybean (53). Moreover, the chemical profiles of various minor components related to health benefits and nutritional quality of products are also affected by fermentation (58). Unlike the production of small molecules by degradation of large ones during fermentation, the content of some compounds is changed by artificial addition during the process (19). Adding salt increases the sodium content in soy paste of doenjang and miso by 9–9.7% and 3.7%, respectively, whereas the potassium content in doenjang, tempeh, and miso is decreased by fermentation. However, chungkukjang and tempeh do not have higher sodium contents because salt is not added (59,60). Although doenjang and miso contain high contents of salt, they have not increased sodium intakes in Asians because they are used as seasoning in place of salt. Thus, doenjang and miso may not increase salt-related hypertension (19).

Fermentation also increases the antioxidant capacity in different products. The study on 30 different fermented soy products revealed that fermentation with different microorganism can improve the antioxidant capacity, vitamin B2, vitamin B12, and GABA (61). A few examples of fermented soy products with varied nutritional values are chungkukjang: protein (41%), lipid (26%), carbohydrate (24%), isoflavones such as genistin, daidzin, glycitin, malonylgenistin, genstein, daidzein, amino acids including aspartate (3.4–3.8%), glutamate (4.4–5.5%), serine (1.5–1.8%), glycine (1.3–1.5%), arginine (1.9–2.1%), alanine (1.5–1.6%), proline (1.8–2.0%), histidine (0.9%), valine (2.0–3.0%), and minerals including Na (0.03%) and K (2.4%). Tempeh: protein

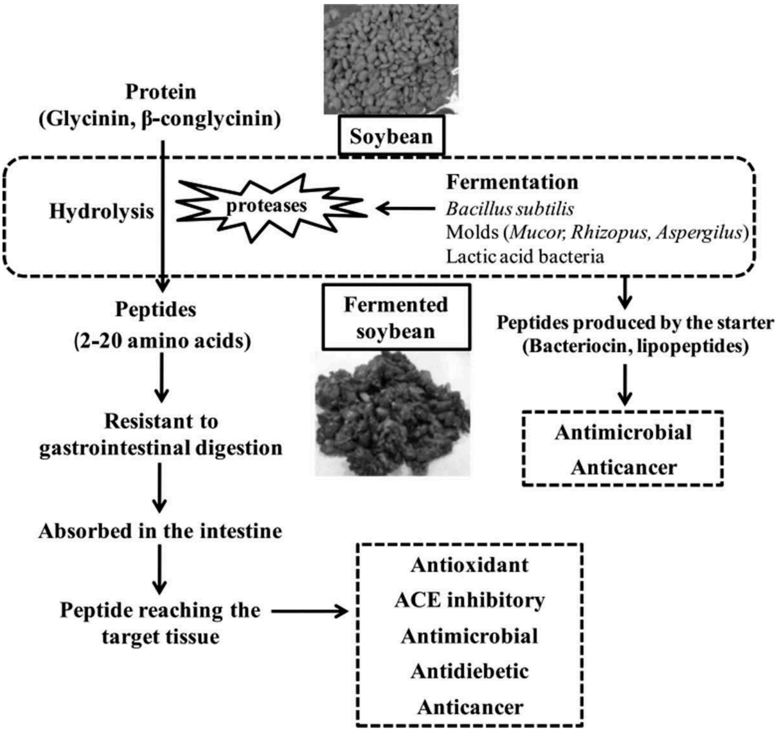
Protein
(Glycinin, β-conglycinin)
Soybean
Hydrolysis
proteases
Fermentation
Bacillus subtilis
Molds (Mucor, Rhizopus, Aspergilus)
Lactic acid bacteria
Peptides
(2-20 amino acids)
Fermented soybean
Peptides produced by the starter
(Bacteriocin, lipopeptides)
Resistant to gastrointestinal digestion
Antimicrobial
Anticancer
Absorbed in the intestine
Peptide reaching the target tissue
Antioxidant
ACE inhibitory
Antimicrobial
Antidiebetic
Anticancer

FIGURE 9.2 Formation and biological activity of bioactive peptides in fermented soybean.
Source: Reproduced with permission of Elsevier (17).

(23–55%), lipid (14–23%), carbohydrate (10–30%), isoflavones such as genistin, daidzin, glycitin, malonylgenistin, genstein, daidzein, amino acids including aspartate (1.5%), glutamate (2.3%), serine (0.8%), glycine (0.6%), arginine (0.9%), alanine (0.6%), proline (0.6%), histidine (0.4%), and valine (0.6%), and minerals including Na (0.0006%) and K (0.4%). Doenjang: protein (12%), lipid (4–11%), carbohydrate (11%), isoflavones such as genistin, daidzin, glycitin, malonylgenistin, genstein, daidzein, amino acids including aspartate (1.2%), glutamate (3%), serine (0.8%), glycine (0.6%), arginine (1.2%), alanine (0.8%), proline (1.2%), histidine (0.4%), valine (0.7%), and minerals including Na (9.1–9.7%) and K (0.8–1%). Miso: protein (10–17%), lipid (3–11%), carbohydrate (15–38%), isoflavones such as genistin, daidzin, glycitin, malonylgenistin, genstein, daidzein, amino acids including aspartate (1.8–4.6%), glutamate (4.9–9.9%), serine (2.0–5.7%), glycine (1.4–4.7%), arginine (0.5–3.7%), proline (2.4–6.4%), histidine (3.0–9.0%), and valine (2.0–5.0%), and minerals including Na (3.7%) and K (0.2%) (18,19).

4 Health Benefits of Fermented Soybean Products

The nutritional value of the fermented soy products gains much attention due to its increased levels compared to the non-fermented ones. Researches have shown that soy products rich in protein could reduce the serum concentrations of total cholesterol, low-density lipoproteins (LDLs), and triglycerides if consumed instead of animal protein. Soy protein has been thought to be responsible for the cholesterol lowering effect of soybean (62,63). However, isoflavones present in soybean having both weak estrogenic and antiestrogenic activity may also partly be responsible for the cholesterol lowering and cardioprotective effects (64). Apart from the lipid-lowering effects, fermented soy products also proved to be effective in attenuating the effects of diabetes mellitus, blood pressure, cardiac disorders, and cancer-related issues. Nutritional values and various health benefits of fermented soy products have recently been reviewed (18). Some in-depth analysis of the research findings are summarized below.

4.1 Antidiabetic Effects

Soybean and other soy products have already shown positive results towards the prevention of diabetes mellitus (DM). It has been reported that soy products rich in isoflavones exhibit the antidiabetic effect. Soybean extract inhibits the glucose uptake into brush border membrane vesicles (65). Since insulin resistance is the hallmark of type 2 diabetes mellitus (T2DM) (66), studies report that soy proteins may improve obesity and diabetes by reducing insulin resistance. The soy protein also shows the effects over the lipid synthesis and breakdown in adipose tissue. It has been suggested that fermented soybean products could be better for preventing or delaying the progression of T2DM, although available data from human studies do not offer complete evidence (19). Chungkukjang is a fermented soy product that has the ability to suppress the effects of diabetes. It can regulate the blood glucose and increase the pancreatic beta-cell functions in C75BL/KsJ-db/db mice. Supplementation of chungkukjang increases the plasma insulin levels, hepatic glycogen content, and hepatic glucokinase activity and shows a significant reduction in the blood glucose levels compared to diabetic mice (67). Glucose homeostasis was maintained in 90% pancreatectomized diabetic rats by supplementation of kochujang, a Korean soy product. Kochujang has been known to decrease body weight, visceral fat, and serum leptin levels, and improve glucose tolerance by enhancing insulin sensitivity; overall results suggest that kochujang can be used as a strong antidiabetic agent by virtue of its antidiabetic property (18,68).

Soy milk is an important soy product that inhibits oxidative stress in T2DM in human trials. The results reveal that fermented soy milk regulates the total antioxidant, 8-isoprostaglandin F2α, malondialdehyde, oxidized glutathione, glutathione peroxidase, and reduced glutathione (GSH), levels. The results of the study conclude that probiotic soy milk consumption could improve oxidative stress in T2DM (69). A recent study evaluated the antidiabetic effects of soybean extract fermented by *B. subtilis* MORI in db/db mice, and found that upon supplementation with the soybean extract, the levels of glycosylated hemoglobin were significantly reduced compared to that of diabetic group. The pancreatic islets architecture was preserved well compared to the diabetic mice and the immunofluorescent intensities of insulin were markedly increased (70). It was evidenced that chungkukjang and meju have insulin secretion capacity. When compared to the non-fermented soybeans, genistein, chungkukjang, or meju extract (fermented for 60 days), treated insulinoma cells (Min6) showed higher insulin secretion capacity (71).

A recent study has shown that fermented paste made from soybean, brown rice, or brown rice in combination with rice bran or red ginseng have significant effects on the antioxidant defense and glucose metabolism on high fat diet fed mice. The fermented paste has regulated the activities of various antioxidant enzymes and also regulated the oxidative stress (72).

The effects of fermented soybean products, isoflavones, and soy peptides on glucose metabolism, insulin secretion, insulin resistance, and β-cell mass were reviewed (19). The glucose tolerance was improved by the enhancement of insulin sensitivity via decrease in adipocytes. Several studies have discussed the effects of kochujang on insulin secretion via glucose stimulation, although some have shown that kochujang supplementation does not modify glucose-stimulated insulin secretion in diabetic rats (18,19).

The combination of 50 g of natto, 60 g Japanese yams, and 40 g of okras was tested on the healthy subjects for duration of 1 day, and results show decreased glucose levels and insulin concentrations and also a proper curve was obtained in the oral glucose tolerance test (73). A dose of 40% chungkukjang has shown an insulin sensitivity and enhanced insulin signaling in liver, and the treatment duration was about 4 weeks in 90% pancreatectomized rats (24,74). A 5% chungkukjang dose administration to C57BL/KsJ-db/db mice for a duration of 8 weeks reduced blood glucose level, glycosylated hemoglobin levels, and improved insulin tolerance and insulin levels (75). A 0.4% water-soluble touchi extract for a duration of 60 days to male KKAy mice has significantly reduced the fasting and post prandial blood glucose levels and enhanced the liver function (76).

4.2 Antioxidant Effects

Oxidative stress occurs as a result of an imbalance between the free radicals and antioxidant mechanism (77). It remains a key mechanism in a variety of diseases including diabetes, cancer, etc. The soybean and its products are efficient in scavenging the free radicals and attenuating the oxidative stress (78).

Antioxidant capacities of 28 different commercially available fermented soy products which include different varieties of douchi, natto, miso, fried yellow soybean sauce, one stinky tofu and moldy tofu were studied. Results suggest that fermented soy products have improved the total phenolic content, total flavonoid content, antioxidant profile, and also brought changes in the isoflavones content. The reason for the changes in the isoflavones may be due to the β-glycosidase activity (79). Free radical scavenging effects of douchi, a traditional soybean food fermented with *Aspergillus* spp., are found to be promising. Several isolated phenols and flavonoids are reported; one of those compounds is 3′-hydroxydaidzein with high DPPH free radical scavenging effects (80). *In vitro* and *in vivo* studies suggested that supplementation of douchi extract increased the levels of various antioxidant enzymes in the liver (81).

4.3 Anticancer Effects

The study with fermented soy milk beverage suggests that fermented soy milk beverage suppressed the *In vitro* proliferation of the human colon cancer cell lines HT-29 and Caco-2 (82). The fermented soy milk inhibited the generation of reactive oxygen species and inhibited the growth of estrogen-receptor positive MCF-7 human breast cancer in mice (83). Rat mammary carcinogenesis was inhibited by fermented soy milk beverage; the soy milk was fermented using beneficial *Bifidobacterium*, which involves the elevation of levels of isoflavones in soy milk. Chungkukjang was found to exhibit the anticancer effects against AGS human gastric adenocarcinoma cells (84). Chungkukjang is a soybean paste that is fermented for only a few days using *Bacillus* spp. It attenuated apoptosis and showed an increased bax and decreased bcl-2 expression of mRNA. A meta-analysis showed that there is an association between soy consumption and prostate cancer risk in men. The reduction in prostate cancer risk in men was seen upon fermented soy products consumption. The results may vary based on the different soy product and its quality. Matrix metalloproteinase plays a significant role in the inflammation and various tumorigenesis processes. In the case of colorectal cancer, increased levels of MMP-9 were observed. The consumption of soybean and its products may have a significant effect on the prevention of cancer and regulation of MMP levels (85). These effects may be due to its high isoflavones content or maybe its phenolic composition (18).

Hepatocellular carcinoma (HCC) is a heterogeneous disease from both molecular and clinical aspect. A recent study found that HCC can be prevented by miso. It has an influence on HCC prevention, and this may be due to its effects on hormonal changes and it also reduced the cell proliferation or it may have a direct effect on angiogenesis and tumor cells (86). In another study, researchers have isolated nine different compounds from miso, and out of those nine, 8-hydroxydaidzein, 8-hydroxygenistein, and syringic acid showed high DPPH-scavenging activity in cancer cell lines (87).

4.4 Anti-inflammatory and Anti-hyperlipidemic Effects

The high n-3 fatty acids content in soy products may play a vital role in the beneficial activity on various low-grade inflammation-related diseases and bioactive components including isoflavones and plant sterol (88,89). A research found that fermented soy milk prevents the cholesterol-induced inflammatory gene expression and improves the fatty acid profile in the skin of rats. Also, proinflammatory cytokines such as interleukin-1β (IL-1β), IL-1α, inducible nitic oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) expression levels were significantly reduced in inflammatory rats upon the soy milk treatment. The results suggest that soy milk can prevent the skin cancer in rats by its anti-inflammatory action. They are accompanied with a change in skin fatty acid composition and reduced macrophages as a result of decreased inflammation (90).

The NF-kB pathway plays an important role in inflammation and it was proved that this pathway was altered by the administration of soy products. Chungkukjang, a fermented soy product of Korea, was proven to possess anti-inflammatory ability (91). The intake of chungkukjang at 400 mg/kg/day significantly reduced anaphylaxis in rats. The anti-inflammatory activity was developed against arachidonic acid-induced mouse ear edema. It has also been reported that soy isoflavones regulate lipid metabolism through an AKT/mTORC1 pathway in diet-induced obesity male rats. Soy isoflavones exhibit remarkable effects on body weight and adiposity, reduce lipid accumulation in the liver, and overall it inhibits mTORC1 via the AKT pathway, resulting in a decreased lipogenesis and adipogenesis, and an enhanced lipolysis and β-oxidation in diet-induced obesity male rats (92). The accumulating evidence proves that soy products rich in isoflavones can act as a good lipid-lowering agent. Doenjang has been reported to lower the cholesterol and lipid oxidation. It can reduce the lipid peroxidation parameters and reduce cholesterol at 33 mg/100 g of the sample (93). Kochujang, a traditional fermented red pepper paste is known for reducing the cholesterol levels. Kochujang also exhibits a strong hypocholesterolemic effect in human trials, and consequently it reduces the total cholesterol levels and low-density lipoprotein-C (LDL-C) levels (94). In addition, the hypocholesterolemic effect of two peptides from soy β-conglycinin in HepG2 cell lines was reported. The two peptides YVVNPDNDEN (peptide 2) and YVVNPDNNEN (peptide 3) show the property similar to that of statins, and it competitively inhibits the hydroxymethylglutaryl-CoA (HMG Co-A) reductase enzyme and inhibits the endogenous cholesterol synthesis and it also, through activation of SREBP2, increases the low-density lipoprotein receptor (LDLR) levels and results in the increased uptake of LDL by liver for degradation (95,96). Soy protein peptides were found to regulate cholesterol homeostasis in HepG2 cell lines. The ability of the protein present in soy to reduce the cholesterol levels could be due to its potential to reduce the production of LDL and this could also act by altering the apolipoprotein B levels in HepG2 cell lines. (97) Fermented kochujang helps to prevent the high-fat diet-induced obesity in mice. A study on the effects of kochujang shows that it can reduce the levels of circulating cholesterol, triglycerides, and blood glucose, downregulate the expression of lipogenic enzymes, and increase the expression of the lipolytic enzymes (98). Koji mould culture was found to inhibit the cholesterol synthesis in hepatic T9A4 cells *In vitro* (99). Nattokinase is an enzyme produced by the fermentation of soybean with *B. subtillis*, shown to possess strong antihyperlipidemic and atherosclerosis protection property. The study involved 76 patients, at the end of the experiment; the plague size, total cholesterol, and total LDL-C were significantly reduced upon the nattokinase oral supplementation. This suggests that natto is effective in reducing the cholesterol and its associated lipids (100).

Few studies have mentioned that soy products could act as a good anti-lipid peroxidative agent. Soybean fermented with *Aspergillus oryzae* shows increased antioxidant levels and inhibits the lipid peroxidation (101). It has been found that natto possesses strong lipid peroxidative and antioxidant effect in cholesterol-fed rats. Natto has influence over the levels of antioxidant enzymes of rats and also has beneficial influence over the thiobarbituric acid reactive substances (TBARS), a potential lipid peroxidation indicator (102).

4.5 Blood Pressure Maintenance

Hypertension is a chronic degenerative disease and is important risk factor for developing other cardiovascular diseases, strokes, renal failure, cerebrovascular accidents, and many other complications (103). The angiotensin-converting enzyme (ACE) plays a fundamental role in blood pressure, as it converts angiotensin I into angiotensin II, a potent vasoconstrictor; it also hydrolyzes the vasodilator peptides bradykinin and kallidin. The inhibition of ACE will cause a vasodilator response, which lowers blood pressure. Therefore, the pursuit for ACE-inhibitory substances, such as peptides, in food has been conducted so that such peptides could be used in the prevention and treatment of hypertension (104). Several ACE-inhibitory peptides have been identified in fermented milk and cheese (104), although their actual antihypertensive mechanism is still unclear (105). Some fermented milks have been demonstrated *in vitro, in vivo*, and in clinical trials to be antihypertensive agents, and their effects in most cases have been attributed to milk peptides (106). A randomized, controlled trial suggests that increased intake of soybean protein may play an important role in preventing and treating hypertension (107).

4.6 Immunostimulatory Activity

Soybean products show some immunostimulatory effects. Soybean mucilage, traditional doenjang and *Bacillus* spp. strains isolated from chungkukjang of Korea, has shown to possess immunostimulatory activity (108). Polysaccharides obtained from chungkukjang also induced mRNA expressions of inducible nitric oxide synthase and tumor necrosis factor-α (TNF-α) by activating nuclear factor B. Therefore it might help to boost the immune system by regulating the associated parameters (109).

4.7 Immunoreactivity Effects

Food allergy is an abnormal immune response to certain food components. Food allergens are typically naturally occurring proteins, with small regions, called epitopes, responsible for the immunoglobulin E (IgE)-mediated allergic response. Soybean is among the so-called ‘big 8’ food allergens, which together account for over 90% of all documented food allergies in the United States (110). Few studies have confirmed the degradation of soybean allergens, particularly GlymBd 30 K, also known as P34, during fermentation by microbial proteolytic enzymes in fermented soybean foods such as soy sauce, miso, tempeh, soybean ingredients and feed-grade soybean meals (111,112). It has also been shown that various lactic acid bacteria are able to hydrolyze α′- and α-subunits of soybean β-conglycinin (113,114).

4.8 Neurostimulatory Effects

Rhizopus oryzae and *Bacillus amyloliquefaciens* were used to synthesize new soy products koji and chungkukjang, respectively, from doenjang. Doenjang showed strong free radical scavenging effects in human hippocampal HT22 neuroblastoma cells. The mixed cultures showed more promising antioxidant activity than the commercial doenjang (115). Kanjang of Korea has also been reported to exhibit neuro protection activity. Indole alkaloids isolated from kanjang showed strong anti-neuroinflammatory action in lipopolysaccharide-induced BV2 microglial cells. The alkaloids decreased the production of nitric oxide and prostaglandin E2 and inhibited the nuclear factor-kB (NF-kB) inflammatory pathway (116).

4.9 Other Health Benefits

Soy and fermented soy products offered several other health benefits such as maintenance of bone health and prevention of osteoporosis and maintained the normal endothelial function. Nattokinase (natto derived) induced fibrin degradation and acted as an alternative therapeutic option in the protection from comorbid asthma (117). It also played an important role in healthy thyroid maintenance, and showed an influence on the developmental effects and fertility.(18)

5 Safety Concerns of Fermented Soybean Products

Fermented soybean products are empirically considered as safe foods, and no systemic epidemiologic studies have so far indicated any public health risk. However, improperly produced or stored fermented soy products lead to potential health risk. Current major concerns are contamination of fermented soy products due to the use of contaminated raw materials, lack of pasteurization, use of poorly controlled natural fermentations, suboptimal fermentation starter cultures, inadequate storage and maturation conditions, and consumption without prior cooking (20).

The microorganisms taking part in fermented soy products have been empirically recognized as safe for a long time. However, in the past two decades, some researchers have found that some of them may produce toxins that contaminate fermented soy products. For example, some *Monascus* strains secrete citrinin (118); *Rhizopus microsporus* can produce two types of mycotoxins—rhizoxins and rhizonins, which thus can be found in tempeh and fermented bean curd (119). *Aspergillus oryzae* can also produce toxic metabolites, such as aspergillomarasmine, cyclopiazonic acid, kojic acid, 3-nitropropionic acid, maltoryzine, and violacetin (120). Although the levels of these toxins are very low in the final product, the safety of these microorganisms and the related fermented soy products should be concerned.

Bacillus cereus could contaminate fermented bean curd, fermented black soybean and fermented soy paste, causing food poisoning. A major outbreak that involved three *B. cereus* strains after consumption of fermented black beans in China, when 139 persons had nausea, vomiting and diarrhea, was reported. Of the three *B. cereus* strains, two were found to produce the emetic toxin cereulide (a heat-labile toxin), which might be the cause of most symptoms of the poisoning (121). Some potential pathogens including Alphaproteobacteria and *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pantoea* spp., *Staphylococcus sciuri*, and *Enterobacter* spp. were detected in fermented black soybeans and dajiang (122,123). Besides the potential pathogens, mycotoxins might also be present in some fermented soy products. The contamination of deoxynivalenol (DON) in domestic and imported soy sauces sold in China was investigated and found that 97.5% of tested samples were contaminated with DON. However, by assuming that a 50 kg individual will consume a 12.20-mL soy sauce serving per day, the exposure dose index of DON for consumption of soy sauce was about 0.034 mg/kg bodyweight/day, which is much lower than the provisional tolerable maximum daily intake of 1 mg/kg body weight/day, according to the JECFA. However, constant intake of DON even in very low content might lead to chronic disease (124).

In addition, harmful bacteria may produce potentially toxic metabolites during fermentation, including nitrogenous compounds known as biogenic amines. Biogenic amines are formed mostly through enzymatic amino acid decarboxylation, as well as reductive amination of ketones and aldehydes (125). Various microorganisms are capable of producing decarboxylases which include *Enterobacteriaceae*, *Bacillus*, *Clostridium*, *Lactobacillus*, and *Pseudomonas* (126). The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine, and agmatine. Ingestion of biogenic amines in high concentrations as well as the deficiency or inhibition of amine oxidases may potentially lead to toxic effects. Though biogenic amines are usually detoxified through monoamine oxidase (MAO) and diamine oxidase (DAO) in the small intestine, potentiation of toxic effects may occur by oversaturation of amine oxidases with high concentrations of biogenic amines, use of monoamine oxidase inhibitors (MAOI), or consumption of alcohol (127,128). Furthermore, biogenic amines may also be converted to potentially carcinogenic N-nitrosamines in the presence of nitrites (127).

Biogenic amine content of fermented soybean products from Asian foods was reviewed to determine whether the food products are safe for consumption. Though the reported ranges of biogenic amine content in fermented soybean foods varied widely, most products contained biogenic amine concentrations at potentially hazardous levels (125). Current research focuses on the development of starter cultures to reduce biogenic amines in fermented soybean foods (129). The use of additives to reduce biogenic amine content in fermented soybean foods has also been reported. The addition of ethanol to the dressing mixture was found to reduce biogenic amine content in prepared sufu (130).

As mentioned earlier, natural fermented soybean products are empirically considered as safe foods. However, 3-Monochloropropane-1,2-diol (3-MCPD) which is one of the chloropropanol group of chemical contaminants is a well-known contaminant of acid-hydrolyzed vegetable protein (acid-HVP). In the traditional production of acid-HVP where defatted vegetable proteins are hydrolyzed by hydrochloric acid, 3-MCPD is formed by high temperature chlorination of residual lipids that are associated with the vegetable proteins. The inclusion of acid-HVP as a savory ingredient to soy sauces or the use of acid treatment in the manufacturing of soy sauces possibly leads to the contamination of the sauces with 3-MCPD. The contaminant has been reported in soy sauces (131,132) as well as in many other foods. 1,3-Dichloropropan-2-ol (1,3-DCP), another chloropropanol, can be formed from 3-MCPD and has been found in soy sauce and related sauces (132,133).

Furthermore, some fermented soy products contain high salt content, e.g. yellow soy sauce contains more than 12% of salt. This technique prevents spoilage and prolongs the product's shelf life. They are typically used as seasonings and soup bases, so the side effects of the high sodium on health are negligible (20). However, not everyone agrees about this. Public health campaigns in several countries encourage population-wide reduced sodium (salt) intake. Excessive sodium intake is independently related to adverse blood pressure and is a key factor in the epidemic of prehypertension/hypertension. In China and Japan, the greatest proportion of dietary sodium comes from salt added during cooking and sauces, including soy sauce and (in Japan) miso. For Japan, most (63%) dietary sodium comes from soy sauce (20%) (134).

6 Conclusions

Fermentation of soybeans can improve the physicochemical and sensory quality of the soy products. Soybean is fermented using bacteria (*B. subtilis* and lactic acid bacteria) and fungi (*Mucor* spp., *Aspergillus* spp. and *Rhizopus* spp.). Fermented soybean products are very popular in many Asian countries. The commonly available soybean products are natto, miso, tofuyo (Japan), douchi, sufu, doubanjiang (China), chungkukjang, doenjang, kanjang, meju (Korea), tempeh (Indonesia), thua-nao (Thialand), kinema, hawaijar, and tungrymbai (India). During fermentation, large protein, lipid, and carbohydrate molecules are broken down by enzymatic hydrolysis to small molecules which are responsible for the unique sensory and functional properties of the final fermented products. It has been proven that soy products rich in protein can reduce the serum concentrations of total cholesterol, LDLs and triglycerides. Apart from these lipid-lowering effects, fermented soy products also proved to be effective in attenuating the effects of diabetes mellitus, blood pressure, cardiac disorders, and cancer-related issues. They are also efficient in scavenging the free radicals and providing some anti-inflammatory, immunostimulatory activity, and neurostimulatory effects. In terms of food safety, fermented soybean products are considered as safe foods. However, improperly produced or stored fermented soybean products lead to potential health risk such as toxin contaminations.

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Fermented Protein-rich Plant-based Foods

Lilis Nuraida
SEAFAST (Southeast Asian Food & Agricultural Science and Technology) Centre and Department of Food Science and Technology, SEAFAST Building Jl. Ulin No. 1, IPB University (Bogor Agricultural University), Kampus IPB Darmaga, Bogor, Indonesia

Tempe

Introduction

Tempe, also spelled as tempeh in English, is an Indonesian origin fermented soybean. Tempe has gained popularity across the world because of its nutritional content, especially as a protein source. It is the only fermented soybean of Indonesian origin, unlike the other fermented soybeans that originated in China or Japan (Shurtleff and Aoyagi 2007). The word tempe is considered to have been derived from Javanese language, from the word 'tumpi', a food made of sagoo and is white in color, which is similar to the color of fresh tempe. There is no written evidence confirming that when actually Javanese people started making tempe. However, tempe has been a part of the Javanese diet, especially in Yogyakarta and Surakarta, since a long time ago, as evident from the semi-historical manuscript Serat Centhini, which states that the Javanese recognized tempe in 16th century (Astuti 1996).

Indonesia is the largest soybean market in Asia and the largest tempe producer in the world. About 50% of soybean is used to produce tempe, 40% for tofu, and 10% for other foods, such as tauco, soy sauce, and others (The Indonesian Standardization Body 2012). Tempe fermentation has been a popular process for several generations. Most of the tempe producers are microscale industries or home industries. According to The Indonesian Standardization Body (2012), there are 81,000 tempe industries producing 2,400,000 tons tempe per year. The data of Central Statistical Bureau showed that the average tempe consumption during 2002–2015 was 7.48 kg/capita/year, and during 2016–2018 was 7.32 kg/capita/year (Ministry of Agriculture Indonesia 2016). Traditionally, tempe is mostly cooked as dishes that are fried, stir-fried, cooked with coconut milk and served together with rice. However, at present, tempe is used to produce a variety of food products, such as tempe chips, tempe burger, tempe nugget, tempe cake, and others. Some of the products such as tempe chips and tempe cake are eaten as snacks. Tempe flour is also being used as an ingredient for the preparation of weaning food.

There are many methods of tempe fermentation, which vary between home industries. Basically, tempe is made from soaked, dehulled, and cooked soybean by inoculating certain mold spores or starter culture called *laru* or *ragi*. The most important mold for tempe fermentation is *Rhizopus oligosporus.* Recent researches (Suwanto et al. 2013, Nurdini et al. 2015) show that other microorganisms such as lactic acid bacteria (LAB) and yeasts are also present in an amount of more than a million per gram during tempe fermentation and they may contribute in improving the quality and safety of tempe. A good-quality tempe has a white compact mass in which the beans are bound together by a mycelium, and when it is sliced the beans are still visible. The standard quality of tempe is regulated in Indonesian National Standard for tempe soybean SNI 3144:2009 of Tempe Kedelai. In 2013, Codex published a regional standard for tempe (Codex Stan 313R-2013). Codex describes tempe as a compact, white, cake-form product, prepared from dehulled boiled soybeans through solid-state fermentation with *Rhizopus* spp.

Fermentation process modifies the organoleptic quality as well as the nutritional value and digestibility of soybean. Nutritionally, tempe represents the food that is rich in proteins and which also contains many other important nutrients, such as vitamin B12 and minerals. The most beneficial effect of soybean fermentation in tempe is improvement in sensory characteristics, nutrition, digestibility, and reduction or inactivation of antinutritional factors (ANFs), such as trypsin inhibitors and phytic acid (Nuraida 2016). Fermentation process also produces and releases some bioactive compounds that are important for health, such as isoflavone aglycon (daidzein, genistein, glycitein) and antibacterial substances. The mixed cultures of fungi and some of the bacterial species are important in degrading complex substances and producing or releasing beneficial compounds during tempe fermentation.

The most common substrate used to make tempe is yellow soybean; hence tempe is referred to as fermented yellow soybean. Various other beans are also be used for tempe fermentation, such as winged bean (*Psophocarpus tetragonolobus*), koro benguk (velvet bean, *Mucuna pruriens)*, koro pedang (red sword bean, *Canavalia ensiformis*; white sword bean, *Canavalia gladiata)*, beludru buncis (*Phaseolus vulgaris*), kacang polong merpati (pigeon pea, *Cajanus cajan*), and others. Tofu solid waste (okara) is also used as a raw material for making tempe. The name of tempe is derived from the raw material used for tempe making, such as tempe koro benguk is made of koro benguk.

Tempe Fermentation

Tempe preparation methods vary between home industries. Saono et al. (1986) described seven different methods of tempe fermentation process. Most of the differences are at preparation steps to make soybean ready for inoculation. Basically, tempe preparation involves soaking, boiling, and inoculating the beans with starter cultures. In some home industries, the beans are boiled before soaking which is then followed by a second boiling step; however, some others prefer soaking the beans before boiling them. In some home industries, the second boiling step is replaced with steaming or simmering the beans in hot water. The difference in the process affects the microbial population during tempe fermentation, which in turn affects the quality of tempe, especially its texture, taste, and aroma.

The Indonesian Stardardization Body (2012) describes that tempe preparation starts with sorting of the soybeans to obtain high-quality filth-free beans. The next step is to wash the beans using running water, followed by boiling them for 30 min. After boiling, the beans are soaked overnight to bring the pH down to below 5. The soaked beans are then dehulled to remove the seed coating, so that the molds are able to penetrate through them. The dehulled beans are washed and then steamed until they are cooked. The cooked beans are then drained and cooled. The cooling stage could be accelerated with the help of a fan. Drained and cooled beans are then mixed with the starter culture. The mixing has to be done thoroughly to spread the spores of the molds. The inoculated beans are then wrapped in banana leaves or packed in perforated plastic bags and incubated at 30°C for 36 h (Figure 10.1). Traditionally, tempe is packed in banana leaf. However, many tempe producers use plastic bags to pack the inoculated beans. When using plastic bags, a perforation should be made to ensure sufficient oxygen supply for the beans.

Each processing step of tempe fermentation contributes to the success of fermentation process. The first boiling step is necessary to reduce the microbial contaminant and to soften the beans prior to soaking process. Soaking process is necessary to lower the pH of the beans to below 5. Tempe fermentation actually consists of two steps. The first step takes place during soaking. Soaking not only softens the beans to make dehulling process easier, but it also acidifies the beans. During soaking, the fermentation takes place spontaneously by microorganisms present in the raw material and in the environment. As in some household industries, soaking step follows the

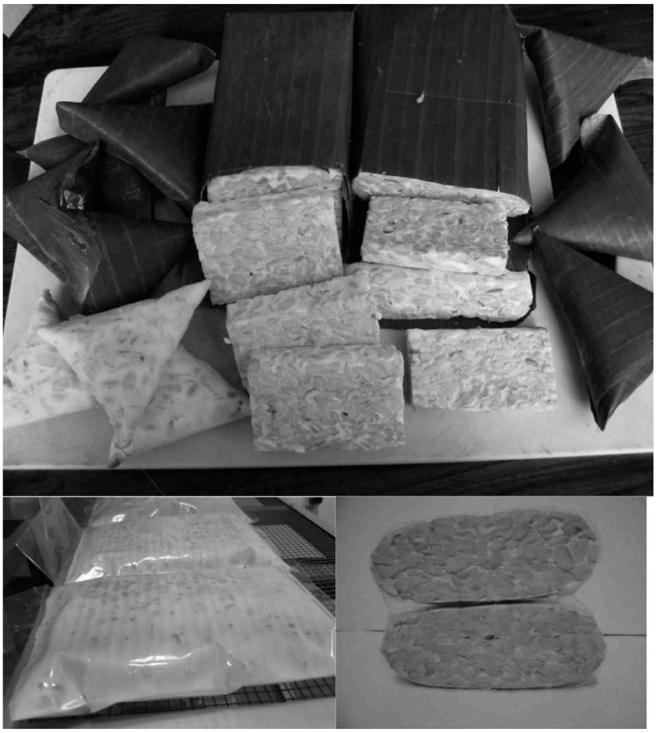

FIGURE 10.1 Tempe wrapped in banana leaf and perforated plastic bag.

boiling step, the acidification during soaking indicates that some bacteria may survive the boiling step. Acidic condition is required to inhibit the growth of spoilage bacteria and to create favorable condition for the growth of tempe mold. During soaking, LAB are present in more than 10^8 cfu/g soybean (Nuraida et al. 2008, Suwanto et al. 2013) indicating that this group of bacteria plays an important role in bringing the pH down. As the fermentation during soaking process is a spontaneous fermentation, the length of soaking process to reach pH below 5 may vary between various home industries. Incomplete acidification may affect the quality and shelf life of tempe. Besides LAB, other bacteria, such as *Bacillus* and *Acetobacter*, have also been found during soaking process (Barus et al. 2008). To control acidification process, pure culture of LAB could be inoculated during soaking process. Acidification using acetic acid has also been tried by some researchers; however, most tempe home industries still rely on natural fermentation for acidification process.

Dehulling the beans is necessary to facilitate penetration of mycelium through the beans. Washing after soaking is done to remove the hull and the slime that is produced during soaking. The second boiling, steaming, or simmering step in hot water that is conducted after soaking is necessary to inactivate the microorganisms that are present during soaking. Draining and cooling are necessary to reduce moisture on the surface of the beans and to lower the temperature. Excessive water on the surface will support the bacterial growth and may spoil the beans during fermentation. Inoculation with active starter culture with sufficient mold spores is an important part in tempe fermentation. The amount of starter culture added should provide sufficient number of spores to initiate fermentation. The mold counts after incubation of tempe, made at home industries, are about $10^{4\ \mathrm{cfu/g}}$ cooked beans (Nurdini et al. 2015). The packaging used for the inoculated beans should permit sufficient supply of oxygen to support the growth of the mold. However, excessive supply of oxygen can also trigger sporulation of the mold, resulting in dark spots. The packaging is also necessary to retain the moisture during fermentation. Other critical factor in tempe fermentation is incubation. The temperature of incubation has to be controlled at 20–35°C for proper growth of the molds. This temperature is the optimal range required for the growth of the molds. During fermentation, free contact of free water with the beans should be avoided; hence excessive evaporation during fermentation should be avoided. Therefore, fermentation room should be well ventilated to avoid excessive heat.

During fermentation, oligosaccharides, fat, and proteins of soybean are degraded by tempe molds. *Rhizopus* sp. produces enzymes such as carbohydrase (polygalacturonase, endosellulase, xylanase, and arabinase), lipase, and proteinase to degrade the carbohydrates, fat, and proteins of soybean (Nout and Kiers 2005). Using single culture of *R. oligosporus*, reduction of raffinose by 60% and stachyose by 10% was observed. In contrast, there were increase in reducing sugar of 700 mg/100 g; and increase in free amino acid of 70 mg/100 g (Egountley and Aworh 2003). The availability of simple compounds supports the growth of bacteria, such as LAB, during tempe fermentation.

Tempe Starter Cultures

Tempe starter culture is called *laru*, which is in the form of cake and powder (Figure 10.2). Laru is made by inoculating steamed rice, moistened rice flour, moistened cassava solid waste, or mixed between rice flour and cassava solid waste with spores of *Rhyzopus oligosporus*, placed in a tray, and incubated at room temperature (25–30°C) until sporulation takes place, which usually takes 3 days. The cakes are then sundried and sometimes powdered. Semi-pure starter culture is prepared by growing a pure culture of *Rhizopus* sp. on cooked, but not sterilized, rice (Nout and Rombouts 1990). Many home industries propagate semi-pure starter culture sold commercially using a cheaper substrate such as cassava solid waste. The cassava solid waste is a by-product of cassava starch production. Propagation is done by inoculating moistened dried onggok with semi-pure starter culture. Moistening of the cassava solid waste is done by using warm water (50–60°C), without any heating process of the substrate (Nuraida and Krusong 2014).

Commercial tempe starter culture with brand name Raprima produced by PT Aneka Fermentasi Industri (AFI) (Bandung, Indonesia) is commonly used for tempe fermentation in Indonesia (Seumahu 2012). The mold that is present in the starter culture of Raprima is *R. oligosporus* (Purwijantiningsih et al. 2005). Other molds are also present in local starter cultures such as *Aspergillus niger, R. oryzae, Rhizomucor pusillus,* and *Mucor rouxii*. Beside molds, spore-forming

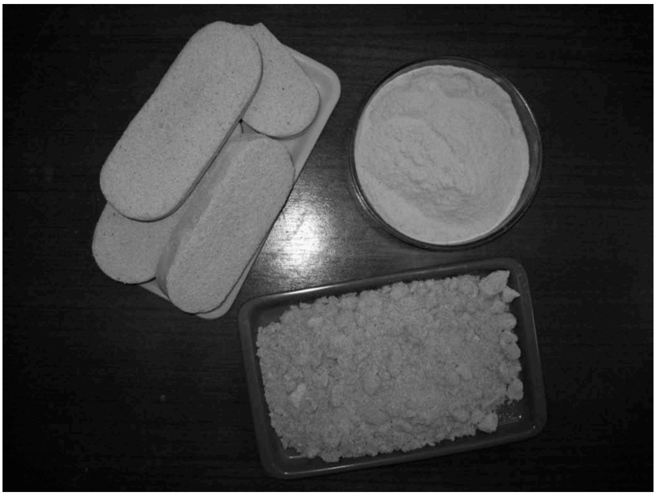

FIGURE 10.2 Tempe starter cultures or laru in the form of cake and powder.

bacteria and *Enterobacteriaceae* in powdered commercial or semi-pure starter culture were also detected by Nurdini (2015). More diverse microorganisms are found in propagated starter culture with cassava solid waste as substrate. LAB and yeasts are also present in sub-cultured *laru*, with total bacteria higher than those in commercial/semi-pure starter culture, i.e., 10^6 cfu/g and 10^5 cfu/g, respectively (Nurdini 2015). Shambuyi et al. (1992) suspected that spores of *Bacillus* and *Clostridium* species are liable to survive and to be activated by the cooking processes used for the preparation of substrates for starter cultures. Ko (1985) reported bacterial concentrations of 10^4–10^5 cfu/g in dried tempe starter cultures.

In Yogyakarta area, tempe home industries traditionally use local starter culture called *usar*. Usar is dried hibiscus (*Hibiscus tiliaceus*) leaf, locally called *waru*, where the hairy underside of the leaf is overgrown by the molds (Nuraida and Krusong 2014). Usar or dried hibiscus leaf overgrown by mold is made by sandwiching inoculated, dehulled, cooked soybeans between the undersides of two hibiscus leaves. The cooked soybean is inoculated with previously made usar or laru. The leaves are incubated for 3–4 days at room temperature (25–30°C) until the bean cotyledons are covered by sporulating mycelium. The moldy beans are removed, and then the leaves are sundried.

Microbiology of Tempe

The main microorganisms used in tempe fermentation are molds, especially *Rhizopus oligosporus.* Other Rhizophus species that have been reported during tempe fermentation include *Rhizopus oryzae, Rhizopus arrhizus,* and *Rhizopus stolonifer.* Other molds present in tempe include *Mucor indicus, Mucor circinelloides, Geotrichum candidum, Aureobasidium pullulans, Alternaria alternata,* and *Cladosporium oxysporum* (Samson et al. 1987). *Rhizopus oligosporus* has the highest proteolytic and lipolytic activities among other molds used during tempe fermentation. This mold is suitable for making tempe by degrading the proteins and lipids in soybean or other substrates. *R. oligosporus* strain that is commonly found in tempe from West Java was strain of NRRL 1526 and is considered as the best strain for soybean tempe fermentation. This strain is commonly found in good-quality tempe (Shurtleff and Aoyagi 1979).

Tempe fermentation is not a totally controlled fermentation; hence a complex community of microorganisms is present during fermentation since soaking process (Nout and Rombouts 1990, Nout and Kiers 2005). Currently, the diversity of bacteria during tempe fermentation is being extensively studied. During soaking, LAB and yeasts are detected (Nuraida et al. 2008, Suwanto et al. 2013, Nurdini 2015). LAB are involved since soaking process and they may be carried over to mold fermentation stage (Nurdini et al. 2015). LAB that are present in high numbers during soaking, acidify the bean under natural fermentation process (Mulyowidarso et al. 1989). Soaking helps in controlling microbial populations in tempe. At the end of the soaking process, *Enterobacteriaceae* could not adapt to the acidic condition because of the organic acids produced by LAB and hence decreases (Mulyowidarso et al. 1989). LAB increases during tempe fermentation and reach 10^6 cfu/g (Nuraida et al. 2008). Higher number of LAB in tempe was reported by Nurdini et al. (2015), in which the number reached to more than 10^7 cfu/g. Touw (2014) identified the presence of *L. fermentum, Lactobacillus delbrueckii* ssp. *delbrueckii, L. plantarum, Weisella confusa,* and *Pediococcus pentosaceus* after soaking. *L. fermentum* and *P. pentosaceus* were also detected in tempe. Meanwhile Seumahu (2008) reported the presence of *Lactobacillus fermentum, Lactobacillus delbrueckii* subsp. *delbrueckii, Lactobacillus delbrueckii* subsp. *bulgaricus,* and *Lactobacillus mucosae* in tempe. Pisol et al. (2015a) isolated LAB from Malaysian tempe and identified as *Leuconostoc lactis* and *Leucontoc* sp.

Yeast develops during fermentation after the boiled beans are inoculated with tempe starter culture (Nurdini et al. 2015). Yeasts are present in high numbers during tempe fermentation and the number increases to 10^8 cfu/g (Nurdini et al. 2015). Nisa (2016) isolated *Candida famata, Cryptococcus laurentii, Candida krusei/inconspicua, Geotrichum klebahnii, Candida pelliculosa, Cryptococcus neoformans, Candida guilliermondii,* and *Candida utilis* from tempe produced by home industries. *Candida krusei/inconspicua* expressed the highest β-glucosidase activity, the enzyme releasing isoflavone agylcon, among the yeasts isolated from tempe (Nisa 2016). *Trichosporon beigelii, Clavispora (Candida)lusitaniae, C. maltosa, C. intermedia, Yarrowia lipolytica, Lodderomyces elongisporus, Rhodotorula mucilaginosa, C. sake, Hansenula fabiani, C. tropicalis, C. parapsilosis, Pichia membranaefaciens, Rhodotorula rubra, C. rugosa, C. curvata,* and *Hansenula anomola* have been reported to be present in tempe in the Netherlands (Samson et al. 2007).

Interaction between molds and other microorganisms in tempe is mentioned in several studies. The growth of *R. oligosporus* is not influenced by LAB (Feng et al. 2005) and yeast (Feng et al. 2007). Nurdini et al. (2015) showed that during the first 12 h, the growth of molds is inhibited. This is probably due to the presence of LAB in high numbers at initial step. High number of LAB at initial step or the significant growth of LAB during the first 12 h seems to inhibit the growth of molds; meanwhile the growth of yeasts is not affected. High number of LAB at initial step is also correlated with low pH at initial step and this may also contribute to inhibition of the growth of *Enterobacteriaceae* and bacterial spores; however, Seumahu (2012) has detected *Enterobacteriaceae* in tempe. On the other hand, bacteria of family *Enterobacteriaceae*, such as *Citobacter freundii* and *Klebsiella pneumoniae*, is responsible for the presence of vitamin B12 and thiamin in tempe (Keuth and Bisping 1993). The vitamin B12 levels in tempe are estimated to be in the range of 2–40 ng/g (Nout and Kiers 2005).

Tempe also contains undesirable microorganisms that can reduce its quality. *Bacillus* grows during tempe fermentation and is considered to cause a bitter taste (Barus et al. 2008). Barus (2008) detected *B. subtilis* in tempe. *Bacillus* sp. exhibits high proteolytic activity for metabolizing protein into amino acids. Seumahu (2012) found *Cronobacter sakazakii* in tempe. Artificially inoculated *C. sakazakii* in tempe at initial concentrations of 1 cfu/g and 3 cfu/g grew well during tempe fermentation without affecting the mold growth, while tempe inoculated with *B. cereus* at 3 log CFU/g resulted in spoiled ones (Dewanti et al. 2017). This experiment indicated that contamination of *C. sakazakii* resulted in good-quality tempe with potential health risk, whereas *B. cereus* contamination produced tempe with unacceptable quality. Other bacteria that have recently been found in tempe include *Acinetobacter baumanii, Acetobacter cibinongensis, Acetobacter pasterianus, Acetobacter indonesiensis, Sphingomonas, Acetobacter* sp. *Azospirilium amazonense, Klebsiella* sp., *Enterobacter* sp., and *Serratia sp.* (Seumahu 2012).

LAB are considered to have a role in controlling pathogenic bacteria during the tempe production. *Lactobacillus plantarum* was reported to have deliberately suppress the growth of *Bacillus cereus* (Ashenafi and Busse 1991a), *Listeria monocytogenes* (Ashenafi and Busse 1991b), and *Salmonella infantis* (Ashenafi and Busse 1991c) during various bean tempe fermentation. The presence of LAB can also inhibit staphylococcal toxin production (Nout and Kiers 2005). Moreno et al. (2002) reported that *Enterococcus faecium* isolated from overripe tempe produced bacteriocin that inhibited the growth of *Listeria monocytogenes*, *Bacillus pumilus,* and *Clostridium sporogenes.* Pisol et al. (2015b) demonstrated that LAB isolated from tempe exhibited varying degrees of inhibitory activities against *E. coli, S. typhimurium*, and *S. aureus.* However, none of the isolates exhibited antimicrobial activity against *Candida albicans* and *Aspergillus niger.*

Nutrition and Health Beneficial Effects

Tempe represents food that is rich in proteins and also contains many other important nutrients, such as lipid, vitamin B12, and minerals. US Department of Agriculture in National Nutrient Database for Standard Reference Legacy Release provided information on nutrition content of tempe, including vitamins and minerals content as presented in Tables 10.1 and 10.2. When soybean is fermented into tempe, the enzymes produced by molds hydrolyze macromolecules of soybean to lower molecular weight substances. Chemical changes during fermentation improve taste, flavor, nutrition, and functional properties of tempe. Proteins are hydrolyzed into peptides, amino acids, and ammonia, resulting in a specific flavor of tempe. Oligosaccharides are hydrolyzed into simple sugars, while phytate is hydrolyzed into organic phosphate. These chemical changes during tempe fermentation increase digestibility of soybean (Astuti et al. 2000). Hermana and Karmini (1996) reported that

TABLE 10.1

Chemical composition of fresh tempe*)

Parameter	Unit	Content per 100 g
Water	g	59.65
Energy	kcal	193
Protein	g	18.54
Total lipid	g	10.80
Total saturated fatty acid	g	2.220
Total unsaturated fatty acid (monounsaturated)	g	3.000
Total polyunsaturated fatty acid	g	3.827

*) US Department of Agriculture.

TABLE 10.2

Vitamin and mineral content of fresh tempe*)

Nutrition component	Unit	Content per 100 g
Vitamin		
Thiamin	Mg	0.078
Riboflavin	Mg	0.358
Niacin	Mg	2.640
Vitamin B6	Mg	0.215
Folate, DFE	µg	24
Vitamin B12	µg	0.08
Mineral		
Calcium, Ca	Mg	111
Iron, Fe	Mg	2.70
Magnesium, Mg	Mg	81
Phosphorus, P	Mg	266
Potassium, K	Mg	412
Sodium, Na	Mg	9
Zinc, Zn	Mg	1.14

*) US Department of Agriculture.

tempe fermentation increases the digestibility of soybean from 75% to 83% and protein efficiency value from 1.6% to 2.2%. Significant increases are also observed in soluble solid, dissolved nitrogen, and free fatty acids content (Hermana and Karmini 1996).

Using different starter culture and soybean cultivars, fermentation into tempe significantly increased the protein content, while decreased the fat content (Bavia et al. 2012, Mustakim 2015), as presented in Table 10.3. Co-culture between mold and bacteria contributes to nutrition of tempe, as well as to the content of bioactive compounds. *Citrobacter freundii* and *Klebsiella pneumoniae* are known to produce vitamin B12 in tempe produced by home industry (Nout and Kiers 2005). The vitamin B12 levels in tempe are estimated to be in the range of 2–40 ng/g (Nout and Kiers 2005). Tempe's molds do not produce vitamin B12 (Keuth and Bisping 1993). As *Citrobacter freundii* and *Klebsiella pneumoniae* synthesize vitamin B12 in tempe, it has become the

TABLE 10.3

Comparison of protein, fat, and ash content between soybean and tempe

		Tempe 1*)		Tempe 2*)	
Nutrition parameter	**Unit (dry weight)**	**Soybean grain**	**Tempe**	**Boiled soybean**	**Tempe**
Protein	%	36.16	43.16	50.41	55.57
Fat	%	23.20	22.13	27.53	23.89
Ash	%	6.13	5.04	1.87	1.57

*) Adapted from Bavia et al. (2012)
**) Adapted from Mustakim (2015)

plant-based food that contains vitamin B12. Besides vitamin B12, *C. freundii* and *Klebsiella pneumoniae* also produce vitamin B6 and riboflavin in tempe (Keuth and Bisping 1993).

Unlike simpler sugars, soybean oligosaccharides such as stachyose, raffinose, and verbacose grouped into galactooligosacharides (GOS) cannot be metabolized by human gastrointestinal (Middlebos and Fahey 2008). Fermenting soybean into tempe also reduces or inactivates ANFs such as trypsin inhibitors and phytic acid (Nuraida 2016). The decrease of phytic acid is very important because its presence inhibits the availability of minerals such as calcium, zinc, and iron.

With better digestibility than soybean, tempe has beneficial physiological effects during malfunction of the gastrointestinal digestive system. Clinical trials in Indonesia showed that the use of tempe in the rehabilitation of children suffering from protein-energy malnutrition had a greater nutritional impact than food mixtures containing cooked but unfermented soybeans (Nout and Kiers 2005).

Tempe is known to exhibit cholesterol-lowering effects because of the presence of protein, fiber, carotenoids, sitosterol, isoflavones, calcium, lecithin, niacin, and/or unsaturated fatty acids in it (Nout and Kiers 2005). Rats that are fed tempe flour had a higher HDL cholesterol as compared to those that are fed soy flour or casein (Astawan et al. 2015). As compared to soybeans, tempe has more health benefits, as during fermentation many bioactive compounds are released or synthesized. Potential health benefits of tempe have been reviewed by Nout and Kiers (2005), which attributed to the presence of glycolipid superoxide dismutase (SOD), a free radical scavenger, and Gamma-aminobutyric acid (GABA) in tempe. Tempe's glycolipid has been demonstrated to inhibit proliferation of tumor cells in mice (Nout and Kiers 2005). GABA in tempe, which is produced by the molds (Handoyo and Morita 2006), has been known to act as an antihypertensive and antiatherosclerosis (Shizuka et al. 2004, Watanabe et al. 2006). The plasma triacylgycerol levels decreased significantly by consumption of GABA-tempe compared with soy protein and casein (Watanabe et al. 2006). The researchers increased the GABA content in tempe by using conventional aerobic fermentation for 20 h followed by anaerobic fermentation for 5 h with *R. microsporus* as a starter culture. Animal experiment showed that SOD activity in serum and liver increased, whereas lipid oxidation decreased in rats consuming tempe (Nout and Kiers 2005). SOD protects cells from the toxicity of free radicals (Astuti et al. 2000). The presence of SOD depends on the growth of the molds.

Fermentation of soybean increases its antioxidative capacity by releasing potent antioxidant substances through transformation of flavonoids to isoflavone aglycons, such as genistein and daidzein, and producing factor 2 (6,7,4¢-trihydroxyisoflavone) (Astuti et al. 2000, Nout and Kiers 2005, Haron et al. 2009). Tempe has the highest amount of total isoflavone (daidzein and genistein) compared to other soy products (Haron et al. 2009). During tempe fermentation, *Rhyzopus* hydrolyses isoflavone glucoside into aglycon (daidzein, genistein, glycitein), which increases the antioxidative capacity of tempe (Nout and Kiers 2005). Bavia et al. (2011) reported that the aglycones content increases about 50% in tempe (49.00 mg/100g) as compared to soybean grains (21.49 mg/100 g). Isoflavone aglycon is also ready to be absorbed by human body. Isoflavone exhibits estrogen-like function, is expected to alleviate symptoms of osteoporosis occurring after menopause, and can suppress the onset of arteriosclerosis, as it also improves the metabolism of lipids such as cholesterol (Nakajima et al. 2005). Isoflavone in the aglycon form has a higher absorptivity than that with the glucoside form. The isoflavone content in tempe is affected by cooking, as shown in a report that deep-fried tempe has 45% lower isoflavone content than that in fresh tempe (Haron et al. 2009).

Several researchers showed that the level and type of isoflavones generated during fermentation depends on the composition of microorganisms as the inoculum. Some LAB and yeasts exhibit β-glucosidase catalyzing conversion of glucoside form to agylcon form of isoflavone. Soybean variety and preparation method of tempe have been reported to affect aglycon form of isoflavone content in tempe. Iskandar and Priatni (2008) reported that tempe made on laboratorium scale has lower isoflavones aglycone content than tempe obtained from the market. However, a study done by Efriwati and Nuraida (2013) found no significant difference in isoflavone aglycon content in tempe obtained from two different home industries in Bogor. However, the results varied when yeast-enriched starter culture was used to make tempe (Nuraida 2016). Co-culture of *Candida pelliculosa* and *L. plantarum* with commercial tempe starter culture increased daidzein and genistein content significantly by 27% and 39% dry base, respectively, as compared to the tempe inoculated with commercial starter culture only. The increase of isoflavone aglycone content was attributed to the presence of *C. pelliculosa*. The presence of LAB did not have significant difference on isoflavone aglycon content (Nuraida 2016). Factor 2 is an aglycon that is only found in tempe among plant-based food. Factor 2 is not synthesized by mold, but by bacteria (Klus et al. 1993, Klus and Barz 1998). Five species of *Micrococcus* and *Arthrobacterv*have have been reported to transform daidzein into factor 2 and other polyhydroxylated isoflavones through hydroxylation of glycitein (Klus et al. 1993, Klus and Barz 1998).

Tempe has been beneficial in reducing diarrhea in the past (Roubos-van den Hil and Nout 2011). Feeding of tempe has shortened the duration of diarrhea in piglet and rabbits infected with *E. coli*. Tempe extracts have decreased the adhesion of most enterotoxigenic *E. coli* (ETEC) tested on piglet brush border cells; hence tempe extracts can prevent intestinal cells being colonized by different strains of ETEC. ETEC is a global cause of severe, watery diarrhea, and is recognized as one of the most frequent causes of childhood diarrhea in developing countries. Reduced adhesion is caused by an interaction between ETEC and soybean compounds. Soya-derived compounds can reduce the adhesion of ETEC to intestinal cells in pigs as well as in humans and can prevent against ETEC-induced diarrheal diseases (Roubos-van den Hil et al. 2009).

The characterization of the antibacterial compounds in tempe showed that after heating, defatting, and protease treatment, the extracts were found to remain active (Roubos-van den Hil et al. 2010a). However, after treatment with polysaccharide-degrading enzyme mixtures, the bioactivity was lost. Further purification of the bioactive tempe extracts yielded an active fraction with an increased carbohydrate content of higher arabinose content than the nonactive fractions. The bioactive components with carbohydrate content are released or formed during fermentation of leguminous matter. The bioactive component contains arabinose and originates from the arabinan or arabinogalactan side chain of the pectic cell wall polysaccharides of the soybeans, which is probably released or formed during fermentation by enzymatic modifications (Roubos-van den Hil et al. 2010a). Soybean tempe extracts strongly inhibited the adhesion of ETEC strains tested. The tempe made from other leguminous seeds were as bioactive as soybean tempe, whereas tempe made from cereals showed no bioactivity (Roubos-van den Hil et al. 2010b). Using soybeans as substrate, fermentation with several fungi (*Mucor, Rhizopus* spp. and yeasts) as well as *Bacillus* spp. resulted in bioactive tempe, whereas fermentation with lactobacilli showed no bioactivity. This effect of soybean tempe is obtained by inhibition of the adhesion of ETEC to intestinal brush border cells (Roubos-van den Hil et al. 2010a). Reduced adhesion of ETEC strains to intestinal cells results in reduced colonization and enterotoxin production, manifested by a lower diarrheal incidence.

Antibacterial activity of tempe extract against *Bacillus cereus* was demonstrated by Roubos-van den Hil et al. (2010c). Cooked beans and *Rhizopus* grown on different media did not show antibacterial activity, indicating the unique association of the antibacterial activity with tempe. During fermentation of soybeans with *Rhizopus*, compounds are released with extensive antibacterial activity against *B. cereus* cells and spores. Characterization of the antibacterial activity revealed that heat treatment and protease addition nullified the bactericidal effect, indicating the proteinaceous nature of the bioactive compound.

Certain species of LAB, such as *L. fermentum*, present in tempe have been reported to be potential as probiotic (Nuraida 2015). A probiotic is defined as a live microorganism that will confer beneficial effects on the host when ingested in sufficient amount (FAO/WHO 2002). Besides having strong antimicrobial activity toward *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus* (Panjaitan et al. 2018), *L. fermentum* isolated from tempe was able to assimilate and bind cholesterol from growing medium (Maryati et al. 2016) hence it has potential to give cholesterol-lowering effect. The potency of LAB with specific functional properties present in tempe is the added value for tempe health beneficial effect.

Non-soybean Tempe

Introduction

Soybean is the largest substrate used to make tempe, and therefore tempe refers to soybean as raw material. In many areas in Central Java, tempe is also made from other beans, including winged bean (*Psophocarpus tetragonolobus*), koro benguk (velvet bean, *Mucuna pruriens)*, koro pedang (red sword bean, *Canavalia ensiformis;* white sword bean, *Canavalia gladiata)*, beludru buncis (*Phaseolus vulgaris*), kacang polong merpati or kacang gude (pigeon pea, *Cajanus cajan*), lamtoro (banana shrubs Selong, *Leucaena leucocephala*), and okara (tofu/soy milk solid waste). When beans other than soybean are used to make tempe, its name is derived from the raw material used (Table 10.4). Some of the nonsoybean tempes are shown in Figure 10.3. Koro benguk is the most common bean used to make nonsoy tempe, followed by koro pedang. Tempe koro benguk has a harder texture and is rough, as the beans are bigger than soybean. In some areas of Central Java, okara (solid waste of soymilk or tofu) and coconut press cake are also used as raw material to make tempe. Nassar et al. (2008) used faba bean, lupine, chickpea, peas, and their mixture for tempe fermentation. Fermentation of those peas with *R. oligosporus* increases protein and fiber content as compared with legume mixtures before fermentation, whereas it lowers the fat, ash, and carbohydrate contents. Lupine has also been investigated for tempe fermentation; however, the molds grew slower than in soybean tempe fermentation, although the sensory characteristic of tempe made from whole lupine beans was not significantly different with that of soy tempe (Priatni et al. 2013). As the characteristics of nonsoy substrates are different than soybean, hence while using them as a substitute for soybean during tempe fermentation, some steps during the preparation of the raw materials requires adjustment.

TABLE 10.4

The local name of tempe made from varieties of beans and other substrates

Raw material	Tempe local name
Winged bean (*Psophocarpus tetragonolobus*)	Tempe kecipir
Koro benguk (velvet bean, *Mucuna pruriens*)	Tempe koro benguk
Koro pedang (red sword bean, *Canavalia ensiformis;* white sword bean, *Canavalia gladiata*)	Tempe koro pedang
Kacang gude (pigeon peas, *Cajanus cajan*)	Tempe kacang gude
Lamtoro (banana shrubs Selong, *Leucaena leucocephala*)	Tempe lamtoro
Okara (tofu/soy milk solid waste)	Tempe gembus
Coconut press cake	Tempe bongkrek

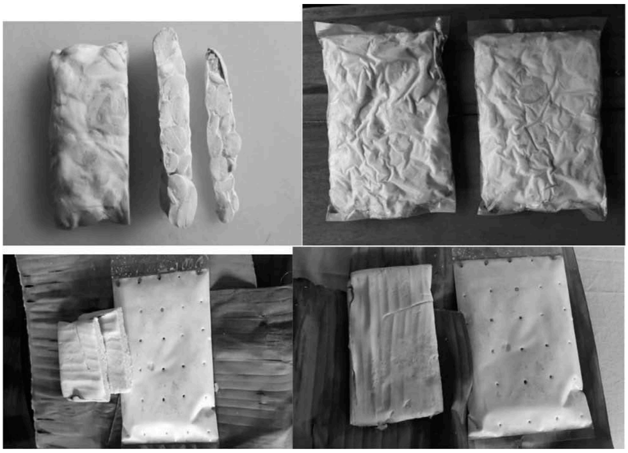

FIGURE 10.3 Tempe koro pedang (jackbeans) and tempe gembus (tempe okara).

Tempe Koro Benguk and Tempe Koro Pedang

Characteristics of Raw Materials

Koro are local beans that are not as popular as soybean. Koro benguk (velvet beans, hyacinth beans, *Mucuna pruriens*) and koro pedang (swordbeans or jackbeans, *Canavalia ensiformis*) are the beans that are commonly used as an alternative raw material to make tempe, especially in Central Java. Jackbeans are also known as Chickasaw lima bean, Brazilian broad bean, coffee bean, ensiform bean, horse bean, mole bean, go-ta-ki, overlook bean, Pearson bean, watanka, and raba de burro (Stephens 1994). *M. pruriens* is native to southeast Asia, but is also currently cultivated in tropical Australia, some regions of the United States, and Latin America (Betancur-Ancona et al. 2012). Velvet beans are used for human consumption in Africa after detoxification (Betancur-Ancona et al. 2012). Forage from *M. pruriens* is fed to cows and sheep, while the seeds can be used in diets for ruminant and nonruminant livestock; it requires no processing for ruminants but must be physically and/or chemically treated to remove ANFs before being fed to nonruminants (Betancur-Ancona et al. 2012). Jackbean has been used mainly as a green manure and animal forage. In human nutrition, Jackbean has more limited uses. Ripe, dry seeds can be eaten, but only after extensive cooking (Betancur-Ancona et al. 2012). Nutritional content of velvet bean and jackbean in comparison with those of soybean is presented in Table 10.5.

Anti-nutritional Factors and Toxic Substances

Legume-based diets are often not effectively used by humans because of their ANFs content. The ANFs interfere in metabolic processes and nutrient availability such that the damage may not be caused by acute intoxication but can result from consuming small quantities over long periods (Betancur-Ancona et al. 2012). Tropical legumes generally contain trypsin and chemyotrypsin inhibitors, phytic acid, tannins, lectins, and cyanogenic glucosides, but individual species may also produce specific ANFs. *C. ensiformis* contains the toxic amino acids canavanine and canaline, which negatively affect digestibility, beside it contains trypsin and chemyotrypsin inhibitors, phytic acid, while *M. pruriens* seed meal is reported to contain nicotine, physostigmine, serotonin, and L-DOPA (Betancur-Ancona et al. 2012). Other toxic substance present in the beans is cyanogenic glucosides. Cyanogens are glycosides of a sugar and a cyanide containing aglycone, and can occur in diverse forms. Its contents in plant vary between species. *C. ensiformis* also contains variable cyanogenic glucoside levels, ranging from 3.02 to 11 mg/100 g (Betancur-Ancona et al. 2012),

TABLE 10.5

Nutritional content of velvet beans, jackbeans, swordbeans, and soybean per 100 g*)

Nutrition	Unit	Velvet beans (*Mucuna pruriens*)	Jackbeans (*Canavalia ensiformis*)	Soybeans (*Glycine Max*)
Water	g	15.0	10.8	12.7
Energy	cal	332	356	381
Protein	g	24	20.5	40.4
Lemak (*Fat*)	g	3	4.1	16.7
Carbohydrate	g	55	61.6	24.9
Fiber	g	5.6	7	3.2
Ash	g	3.0	3	5.5
Ca	mg	30	150	222
P	mg	200	272	682
Fe	mg	2.0	6.2	10
Na	mg	42	18	210
K	mg	389.2	301.8	713.4
Cu	mg	1.25	0.73	1.58
Zn	mg	8.7	2.8	3.9
Beta-Carotenes	mcg	0	0	237
Total carotenes (Re)	mcg	70	NA	31
Thiamin (Vit. B1)	mg	0.30	0.29	0.52
Riboflavin (Vit. B2)	mg	0.12	0.02	0.12
Niacin	mg	1.7	1.4	1.2
Vitamin C	mg	0	1	NA

*) Ministry of Health Indonesia (2017).

while in *M. pruriens* ranging from 5.1 to 10.8 mg/kg (Hamzah and Hamzah 2011). Cyanogens can be hydrolyzed by enzymes to release HCN. L-DOPA (L-3,4-dihydroxyphenylalanine) that is present in *M. pruriens* is a nonprotein amino acid precursor of the dopamine transmitter. Dopamine content in the brain is reduced by blocking of sulfur as it passes through blood boundaries, preventing it from reaching its action site. L-DOPA crosses these boundaries and converts into dopamine, thus continuing neurotransmission. The compound is used to treat the symptoms of Parkinson's disease; however, treatment with this compound can cause serious hallucinations and gastrointestinal disorders such as nausea, vomiting, and anorexia (Betancur-Ancona et al. 2012).

Effective processing techniques are needed to ensure optimal ANFs deactivation and/or reduction. Both nonthermal processes, such as imbibition, germination, dehulling, and fermentation, and thermal processes include cooking at ambient atmospheric pressure, cooking under high pressure (autoclaving) and toasting could lower 40–98% levels of ANFs as well as increase *in vitro* protein and carbohydrate digestibility (Betancur-Ancona et al. 2012). Soaking process for 24–28 h with replacing soaking water regularly (every 6–8 h) reduces glucocyanide from the beans. HCN content in fresh beans was 11.05 mg/100 g and after soaking for 3 days decreased to 0.3 mg/100 g (Kasmidjo 1990). HCN is not detected in tempe made of *M. pruriens* (Hamzah and Hamzah 2011). Boiling also reduces toxic substances from the beans (Stephens 1994). Akinmuntimi and Ukpabi (2008) reported that boiling *M. pruriens* for 90 min reduces antinutritional factors by 100% for trypsin inhibitor, 69.62% for hydrogen cyanide, and 27.05% for tannin. Soaking for 72 h followed by boiling for 30 min reduce the HCN content in Jackbean by 78% and the HCN content in tempe made of the beans was 1.2 ppm (Arpah et al. 2015).

Fermentation

Traditionally, preparation for making tempe *koro benguk (M. pruriens*) starts with cleaning the beans, boiling for 2–3 h, followed by soaking for 2–3 days with regularly replacing soaking water to reduce the toxic substances. The seeds are then mashed in a bamboo or wood container to reduce the size to about soybean size. The mashed beans are then washed and steamed, followed by cooling. The next step is similar to soybean tempe fermentation, i.e., inoculation with tempe starter, wrapping, and incubation. Other variation is applying two-stage cooking and soaking of dehulled and coarsely ground *M. pruriens*. After overnight soaking, the beans are boiled and re-soaked overnight in fresh water followed by re-boiling. Similar process of tempe preparation is applied when using *C. ensiformis* as raw material, except that the beans are not mashed or ground.

Tempe Gembus

Characteristics of Raw Materials

Tempe gembus is made from okara that is a by-product from the soymilk or tofu industries. Raw okara, which has a white yellowish color, consists of the insoluble material from soybean seeds that remain in the filter sack when pureed soybeans are filtered for the production of soymilk. Okara is enriched in cell wall polysaccharides (Mateos-Aparicio et al. 2010) and contains mostly crude fiber composed of cellulose, hemicellulose, and lignin, about 25% protein, 10-15% oil, but little starch or simple carbohydrates (O'Toole 1999). Okara exhibits several health effects, such as a potential source of antioxidant components and bioactive peptides, as a weight-loss dietary supplement, and possesses prebiotic effect (Mateos-Aparicio et al. 2010).

Fermentation

To make tempe gembus, fresh okara is washed to reduce the acidity and pressed/squeezed using a filter fabric to remove excessive water. This step is repeated twice. The pressed okara is steamed for 30 min and then transferred to a tray for cooling. When the temperature reaches room temperature, the steamed okara is inoculated with tempe starter culture, and then mixed thoroughly and wrapped in banana leaf or perforated plastic bag. The incubation is done at room temperature (25–30°C) for 36–48 h. The texture of okara tempe or tempe gembus is softer than soybean tempe or any other bean.

Nutrition

Tempe koro benguk, koro pedang, and tempe gembus are considered as sources of protein (Table 10.6), although their protein content is lower than that of soybean tempe. As it happens during soybean fermentation into tempe, fermentation of nonsoybean legumes improves their nutritional quality and provides edible products with palatable sensorial characteristics.

Tempe Bongkrek

Tempe bongkrek is made of coconut pressed cake or coconut milk residue. The process to make tempe bongkrek is similar to that of tempe gembus described above. Tempe bongkrek is less popular, as consumption of tempe bongkrek is often associated with a food-borne human intoxication resulting in significant numbers of deaths. The causative organism for the intoxication is the bacterium *Burkholderia cocovenenans* that produces two toxins, toxoflavin and bongkrekic acid (also commonly referred to as bongkrek acid) (Garcia et al. 1999). Later *B. cocovenenans* is classified as *Bulkholderia gladioli* pathovar *cocovenenans* to differentiate from other phytopathogenic pathovars within other *B. gladioli* (Jiao et al. 2003). These bacteria contaminate raw material and grow together with tempe mold. *B. cocovenenans* proliferation and toxin production increase when these food products undergo incomplete fermentation

TABLE 10.6

Nutritional content of tempe koro benguk and tempe gembus per 100 g

Nutrition	Unit	Tempe koro benguk (*Mucuna pruriens*)*)	Tempe koro pedang (*Canavalia enciformis* L.)**)	Tempe Gembus (Okara)*)
Water	g	64	60.6	81.9
Energy	cal	141	NA	73
Protein	g	10.2	10	5.7
Lemak (*Fat*)	g	1.3	1.7	1.3
Carbohydrate	g	23.2	NA	10.3
Fiber	g	NA	1.5	4.2
Ash	g	1.3	0.3	0.8
Ca	mg	42	NA	204
P	mg	15	NA	80
Fe	mg	2.6	NA	1.5
Total carotenes (Re)	mcg	NA	NA	22
Thiamin (Vit. B1)	mg	0.09	NA	0.09
Riboflavin (Vit. B2)	mg	NA	NA	0.1
Niacin	mg	NA	NA	0.9

*) Ministry of Health Indonesia (2017). Data of Food Composition Indonesia.
**) Adapted from Ganjar et al. (1979).
NA: Data not available.

(Anwar et al. 2017). Bongkrekic acid is odorless and tasteless; affected food products can have a normal appearance, smell, and taste. Research by Garcia et al. (1999) demonstrated that the concentration and type of lipid in the substrate is critical for bongkrekic acid formation. Of eight saturated fatty acids tested, only lauric (12:0), myristic (14:0), and palmitic (16:0) acids stimulated the production of detectable amounts of toxin. When four 18-carbon free fatty acids with different degrees of saturation were compared, significant amounts of bongkrekic acid (2.62 mg/g dry weight) were produced only with oleic acid (18:1).

Oncom

Description

Oncom is another high-protein vegetable-based traditional Indonesian fermented food. Oncom is mainly produced and consumed in West Java. As oncom is made mainly from agricultural waste product, the price of oncom is cheaper than that of soybean tempe. There are two types of oncom, i.e., red oncom and black oncom (Figure 10.4). The raw materials of oncom are mainly the waste of agricultural products, especially peanut press cake (solid waste of peanut oil) and okara (solid waste of soymilk or tofu). For both types of oncom, tapioca solid waste is used as a mixture of raw materials. The difference between black oncom and red oncom is the type of mold used as starter cultures. Black oncom is made by using tempe starter culture containing *Rhizopus,* particularly *R. oligosporus,* with peanut press cake as the main raw material. Red oncom is made by using *Neurospora*, particularly *N. crasa, N. intermedia,* and *N. sitophila,* with peanut press cake or soymilk solid waste (okara) as raw material. *N. intermedia var. oncomensis* has bright yellow colored large macroconidia (Hoo 1986).

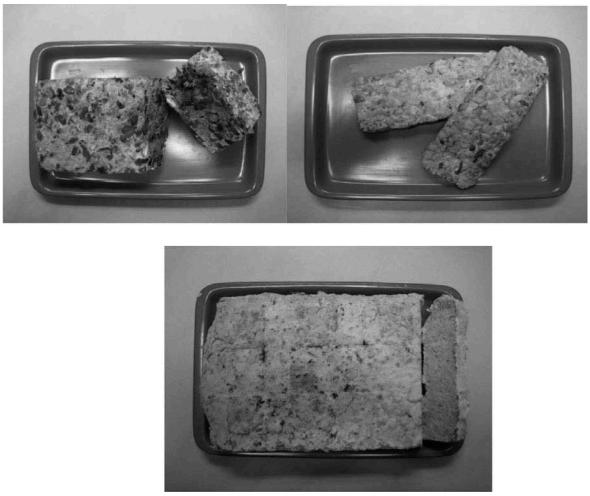

FIGURE 10.4 Red oncom made of peanut press cake, black oncom made of peanut press cake and red oncom made of tofu solid waste or okara.

Fermentation

To make red oncom from okara, the preparation is similar to that of tempe gembus/tempe okara as described above; however, before steaming, the okara is mixed with tapioca solid waste. In contrast to tempe fermentation, for making red oncom of okara, the previous oncom is used as a starter culture. After inoculation, the inoculated okara is placed in a bamboo tray that is lined with banana leaf or paper, and also covered with banana leaf or paper and then incubated at room temperature for 36–48 h.

When peanut press cake is used as raw material to make oncom, the peanut press cake is soaked overnight to separate residual peanut oil from the cake. To bring pH to 4.5 faster, some home industries add lactic or acetic acid into soaking water. The cake is then pressed to remove excessive water, mixed with tapioca solid waste, and then steamed for 30 min. The mixture is then kept for cooling and is inoculated with previous red oncom, for making red oncom, or with black oncom or tempe starter culture, to make black oncom. The flavor of fresh peanut oncom is strong, fruity, almond-like, and somewhat alcoholic. Desirable flavors are developed during the fermentation process (Beuchat 1976).

Nutrition Content

Nutrition of peanut oncom is presented in Table 10.7. The overall nutritive value of protein and lipid appears to have changed only slightly during the fermentation of peanut press cake. Increases in riboflavin, niacin, and possibly thiamin in the finished product are noted (Beuchat 1976). In tempe, enzymatic hydrolysis of peanut constituents greatly enhances its edibility and, presumably, the digestibility of peanut press cake. The PER of the peanut press cake fermented by a mixed culture was 1.41; replacing 10% of peanut protein with sesame protein raises the PER of the fermented product to 2.11 (casein PER = 2.50) (Fardiaz and Markakis 1981a). During fermentation, phytic acid in peanut press cake (1.36% on a dry basis) decreased rapidly (Fardiaz and Markakis 1981b). Oncom prepared with *R. oligosporus* contained the least amount of phytic acid (0.05% after 72 h of fermentation), while oncom prepared with the Indonesian strain of *Neurospora* contained the largest amount of it (0.70%).

TABLE 10.7

Nutrition content of peanut oncom per 100 g

Nutrition	Unit	Quantity
Water	g	57.0
Energy	cal	187
Protein	g	13.0
Lemak (*Fat*)	g	6.0
Carbohydrate	g	22.6
Ash	g	1.4
Ca	mg	96
P	mg	115
Fe	mg	27.0

*) Ministry of Health Indonesia (2017).

Tauco

Introduction

Tauco is an Indonesian style miso, which is a product of fermented yellow soybean paste and is used as a condiment, especially used for Chinese-Indonesian and Sundanese cuisine, such as vegetable soups, meats, and fish to enhance their flavor. Similar products to tauco are chiang in China, jang or doenjang in Korea, and tao chieo in Thailand. The word tauco originated from China and its name comes from its pronunciation in the Hokkien dialect of the Chinese language (Surono 2016). Originally, Chinese make douchi or tausi, a traditional salted and fermented black soybean (Anonym 2018); however in Indonesia, they make tauco from yellow soybean. The *douchi* sauce is a rich source of protein and is also used as a flavored ingredient for cooking, similar to miso (Mani and Ming 2017). Unlike tempe, tauco's popularity is limited to Chinese-Indonesian or Sundanese. The biggest tauco producer is Cianjur, a city in West Java. Tauco is also produced in Bangka, Medan, and Pekalongan in lesser

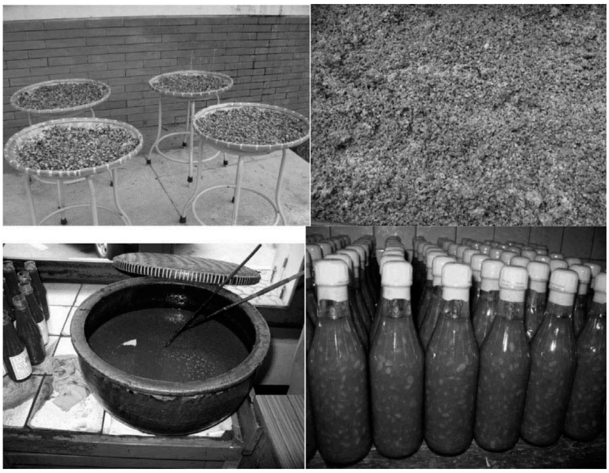

FIGURE 10.5 Drying molded soybeans for tauco fermentation, the salted beans for brine fermentation and tauco paste ready to use as seasoning.

amount. There are two types of tauco, slurry or paste tauco and dry tauco (Figure 10.5). Slurry or paste tauco especially produced in Cianjur, whereas dried tauco is produced in Pekalongan. The tauco paste is made by cooking freshly fermented tauco with palm sugar, and then packed in bottles. To make dry tauco, the paste is sundried.

Fermentation and Microbiology of Tauco

Fermentation of tauco is similar to the fermentation of douche and miso. Two steps are involved in preparing tauco, i.e., mold fermentation and brine fermentation. The fermentation process of douchi involves fungal solid-state fermentation (pre-fermentation) with microorganisms (e.g., *Aspergillus, Mucor, Rhizopus*, bacteria), followed by salting and maturation (post fermentation) (Mani and Ming 2017). The molds involved in tauco fermentation consist of *Rhizopus oligosporus, R. oryzae*, and *Aspergillusoryzae* (Surono 2016). In some home industries, the starter culture of mold fermentation for making tauco is similar to that used for tempe. The mold fermentation or koji fermentation is started with soaking the beans overnight in fresh water, followed by dehulling, boiling, draining, and cooling them on bamboo trays. The beans are mixed with roasted rice flour and inoculated with starter cultures made of previous batch of mold fermentation. In some home industries, the mold fermentation takes place without inoculation. The beans on bamboo trays are placed in incubation room that has been saturated by the mold spores from pervious batches. Fermentation takes place for 3–5 days. On completion of mold fermentation, the cake is crumbled and sundried followed by soaking in brine (20% salt solution) for 20–30 days. Traditionally, brine fermentation is done in earthenware covered with bamboo tray. However, at present the brine fermentation is done in plastic drums. The brine fermentation involves LAB and yeast, namely *Lactobacillus delbrueckii, Hansenula* sp., and *Zygosaccharomyces soyae* (Surono 2016). During brine fermentation, the slurry is regularly mixed to avoid re-growth of the molds and incubated

TABLE 10.8

Nutrition content of tauco per 100 g*)

Nutrition	Unit	Paste tauco	Dry tauco
Water	g	54.0	11.4
Energy	cal	184	347
Protein	g	11.4	7.4
Lemak (*Fat*)	g	5.5	5.2
Carbohydrate	g	22.2	67.6
Fiber	g	1.9	3.2
Ash	g	6.9	8.4
Ca	mg	72	63
P	mg	463	106
Fe	mg	6.0	6.2
Natrium (Na)	mg	1,766	NA
Kalium (K)	mg	233.0	NA
Cu	mg	0.39	NA
Zn	mg	1.0	NA
Total carotenes (Re)	mcg	664	936
Thiamin (Vit. B1)	mg	0.32	0.35
Riboflavin (Vit. B2)	mg	0.20	NA
Niacin	mg	0.8	NA

*) Ministry of Health Indonesia (2017). Data of Food Composition Indonesia.
NA: data not available.

under the sun during the day. The brine fermentation takes place for 3–4 weeks. During fermentation and aging, soybean protein is digested by proteases produced by *Aspergillus oryzae* in the koji. Amino acids and their salts, particularly sodium glutamate, contribute to flavor (Nout et al. 2007).

On completion of brine fermentation, the slurry is mixed with palm sugar solution (25%) and cooked until the soybean mass forms a paste. The paste is bottled and ready for sale. In some home industries, some spices are also added to the slurry while cooking. To make dry tauco, the cooked slurry is spread on bamboo trays and sundried. Nutrition content of tauco is depicted in Table 10.8.

Soy Sauce

Introduction

Soy sauce is a dark brown liquid, stable at ambient temperature, which does not require refrigeration during storage because of its low water activity and high salt content. It is used as a condiment or seasoning in preparation of dishes. Soy sauce is widely consumed in China, Japan, Korea, and many others Asian countries, including Indonesia. The most known type of soy sauce is the salty soy sauce, which possesses mainly salty and *umami* taste with appetizing aroma. This soy sauce can be grouped further into two types, i.e., a Japanese type (only found in Japan) and a Chinese type (produced in China and South East Asia) (Apriyantono et al. 1999). The Japanese soy sauce uses soybeans and wheat as the main raw materials, whereas the Chinese type uses no or very little wheat besides soybeans. Soy sauce in Indonesia is called kecap. There are two types of soy sauce, i.e., salty soy sauce or kecap asin and sweet soy sauce or kecap manis. Kecap manis is a typical Indonesian soy sauce and is more popular than salty soy sauce. Unlike salty soy sauce, the taste of kecap manis is mainly sweet, tasty, and umami with a typical aroma. The Maillard reaction plays a major role in the formation of volatiles and color of kecap manis involving coconut sugar that is added during processing of sweet soy sauce (as a source of sugar) and moromi (as a source of amino acids and peptides) (Apriyantono et al. 1999).

Fermentation and Microbiology of Soy Sauce

Similar to tauco, soy sauce is made by a two-step fermentation process, i.e., mold or koji fermentation or locally called *bungkil* followed by brine fermentation or moromi, locally called *baceman*. Traditional kecap production only uses black soybean as raw material; however, industrial manufacturers use defatted yellow soybean flakes and wheat in addition to black soybeans (Roling and van Verseveld 1996). After the brine fermentation, the soybean mash or moromi is pressed off and filtered. To make salty soy sauce, the filtrate is clarified. To make sweet soy sauce, the filtrate is boiled for 1 h with palm/coconut sugar, molasses, and spices, followed by packaging in bottles or plastic pouch.

Mold Fermentation

In some home industries, mold fermentation takes place spontaneously, as the incubation room is been used over and over without cleaning so it is saturated with mold spores. In some others, molded beans from previous batch of mold fermentation are used as starter cultures. The (inoculated) beans are spread on the trays and then placed in a semi-dark room at ambient temperature (28–32°C) (Judoamidjojo et al. 1985). Yellowish green and gray-colored microorganisms grow on the surface of the spread soybean. In some home industries, the molded beans are dried prior to brine fermentation. The molded beans are then immersed in brine solution with a final concentration of 18–20% in an earthenware barrel or a plastic tank. The tanks are opened during the day time and exposed to sunlight but are covered at night (Judoamidjojo et al. 1985).

During mold or koji fermentation, the mold grows and releases enzymes that break down the proteins and oligosaccharides present in the substrate (Yong and Wood 1974). The mold produces proteolytic and amylolytic enzymes (Luh 1995). A good culture starter must give characteristic aroma and flavor to the soy sauce, have high proteolytic and amylolytic activities, and must be easy to culture. The second stage begins when the mold growth reaches a desired level. The material is then added to a salt solution (18–20% salt), and an anaerobic fermentation is initiated, in which lactobacilli and yeasts predominate (Yong and Wood 1974).

Prior to mold fermentation, the soybean undergoes soaking and cooking. Acidification during soaking inhibits bacterial growth during the solid-state fermentation, causing lower initial numbers of bacteria and a slower acidification of the brine fermentation (Roling et al. 1994a). Cooking time influences the softness of the beans and thus fungal growth during the koji fermentation or *bungkil* stage. Increasing the cooking time from 1 to 3 h resulted in a better solubility of macromolecules and gave higher final concentrations of fermentation products and formol nitrogen in brine fermentation. The highest amounts of formol nitrogen were found with 5 days of mold fermentation (Roling et al. 1994a). The growth of fungi during the mold fermentation stage was also positively influenced by the addition of rice flour. Sun-drying and crumbling of mold-fermented soybeans caused lower enzyme activities and resulted in lesser amounts of formol nitrogen and glycerol (Roling et al. 1994a).

Brine Fermentation

While mold fermentation takes place for 2–7 days, brine fermentation takes place for up to 4 months (Roling and van Verseveld 1996). Shorter brine fermentation period of 4–6 weeks was reported by Judoamidjojo et al. (1985). To lower the production costs, several attempts were made to shorten the long fermentation period required to produce a relatively good soy sauce and to obtain a product of more constant quality at the same time (Yong and Wood 1974).

Roling et al. (1994b) studied the brine fermentation of different *kecap* producers. They found that the final composition of the brine fermented beans (*baceman)* differed from manufacturer to manufacturer, and even within companies large differences were found in microflora and the amounts of fermentation products, formol nitrogen, and salt concentration. The main fermentation products were lactate, acetate, glycerol, and ethanol. *Pediococcus (Tetragenococcus) halophilus*, staphylococci, a coryneform bacterium and yeasts belonging to *Candida, Debaromyces,* and *Sterigmatomyces* were isolated from the brines. Compared to Japanese soy sauce production, fermentation by yeasts does not play an important role in Indonesian *kecap* production. This is because of the fact that Indonesian *kecap* is made from whole soybeans only, which are poor in sugars. After fermentation by *Tetragenococcus halophilus*, no substrates are left for growth and ethanol production by yeasts. The presence of film-forming yeasts can even lead to spoilage of the product (Roling et al. 1994b). Growth of *T. halophilus* during the brine fermentation stage was faster when molded beans were sundried; although the final concentrations of fermentation products were lower due to the lower content of amino acids, which resulted in less buffering. Increasing the amount of molded beans added to salty water caused a similar increase in formol nitrogen and fermentation products (Roling et al. 1994a).

Three overlapping phases could be distinguished in brine fermentation in industrial manufacturing (Roling and van Verseveld 1996), i.e., amino acid production, followed by fermentation by LAB, and yeast fermentation. Amino acid production starts directly after the preparation of brine. In general, amino acids are produced during the first 3 weeks. The amino acid mainly responsible for flavor, glutamic acid, is produced during a longer period (Roling and van Verseveld 1996).

A number of salt-sensitive bacteria, such as staphylococci and *Enterobacteriaceae*, derived from mold fermentation, decreased rapidly after the addition of salt. Only *Bacilli* could be observed after 1 week. The numbers of salt-tolerant bacteria were high at the start of fermentation (10^{7-8} cfu/ml), but dropped rapidly within 2 days before increasing again. The high initial numbers of salt-tolerant bacteria were caused by staphylococci derived from mold fermentation stage. After 1 week of brine fermentation, only the lactic acid bacterium *T. halophilus* was isolated from samples, its maximum number reached around 10^8 cfu/ml. The actual fermentation started after 5–10 days, and during the growth of *T. halophilus*, pH of the brine dropped from 5.0–5.1 to 4.4–4.6. Lactic acid fermentation took about 2.5–3 weeks. Immediately after the preparation of brine, high numbers of yeasts were observed (10^{4-5} cfu/ml). During yeast fermentation, a slight increase in yeast number as well as in ethanol and glycerol concentrations was observed. *Zygosaccharomyces rouxii* was the dominant yeast species (Roling and van Verseveld 1996).

Despite the fact that the brine is not inoculated, microbial and biochemical changes at the Indonesian industrial manufacturer resemble the soy mash fermentation in Japan, i.e., amino acid is produced, and then fermentation by the lactic acid bacterium *T. halophile* is followed by fermentation by the yeast *Z. rouxii.* Owing to the tropical weather, both formol nitrogen production and microbial growth are faster than those during soy sauce production in Japan, which are executed in a moderate climate (Roling and van Verseveld 1996). For good-quality production, a consistent composition of brine extract is required. In traditional *kecap* production, large variations in final composition of batches from the same manufacturer were observed (Roling and van Verseveld 1996). *Z. rouxii* produces flavor compounds, including alcohols, glycerol, esters, and furanones. The 4-hydroxy-2 (or 5)-ethyl-5(or 2) -methyl-3(2 H)-furanone (HEMF) produced by *Z. rouxii* gives Japanese-type soy sauce its characteristic flavor, and is also reported to have antitumor and antioxidative properties (Nout et al. 2007).

Sugiyama (1984) reported that microorganisms related to soy sauce production are molds (*Aspergillus oryzae* or *Aspergillus sojae*), involved in the first step of fermentation (mold fermentation) and LAB (*Pediococcus (Tetragenococcus) halophilus*) and osmophilic yeasts (*Saccharomyces rouxii, Candida (Torulopsis) versatilis, Candida (Torulopsis) etchellsii*), involved in the second step of fermentation. *Aspergillus oryzae* or *Aspergillus sojae* do not produce mycotoxins, and possesses high proteolytic, amylolytic, and macerating enzyme levels. *T. halophilus* produces lactic acid and other useful organic acids in a high concentration of salt brine, and does not possess tyrosine decarboxylase and histidine decarboxylase. *Saccharomyces rouxii* produces alcohol and excellent flavor substances in a high salt concentration brine, while *Candida (Torulopsis) versatilis* and *C. etchellsii* produce excellent flavor substances in a high salt concentration brine (Sugiyama 1984).

Conclusion

Fermentation of protein-rich plant-based foods has not only improved sensory characteristics, but also has improved the digestibility and nutritional quality through enrichment of food substrates with vitamins, proteins, essential amino acids and essential fatty acids, and destructed the ANFs and toxic substances. During fermentation, some bioactive compounds are also released, such as antioxidants and antimicrobial compounds. Therefore some fermented protein-rich plant-based foods, especially tempe may be considered as functional foods as they have additional health benefits beyond supplying the basic nutrition. The microorganisms involved in fermentation may be are exploited to increase the content of bioactive compounds in fermented foods. LAB in fermented food are also promising in promoting health benefits. Therefore the potential of protein-rich plant-based foods as functional foods needs to be investigated further.

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Fermented Soybean Food Products as Sources of Protein-rich Diet: An overview

Adikesavan Selvi
Environmental Molecular Microbiology Research Laboratory, Department of Biotechnology, Thiruvalluvar University, Vellore, India

Nilanjana Das
Bioremediation Laboratory, School of Biosciences and Technology, VIT, Vellore, India

Introduction

Fermented foods are of great significance and are produced with the help of microbes or microbial enzymes, resulting in significant biochemical alteration and desirable food modification characteristics (Campbell-Platt 1994). In general, the fermented foods are considered safe for consumption and possess good safety even in the developing countries. Recent research works on identification and characterization of the bioactive components formed during fermentation are gaining focus, owing to the health benefits of fermented foods. As fermented foods show no side effects, they can be used for the treatment of various diseases. Nutritional value of the fermented foods is attributed to (1) probiotics (presence of live organisms), (2) production of biocompounds by fermenting microbes (peptides, exopolysaccharides, and so on), and (3) production of bioactive compounds during fermentation (peptides, free polyphenols) (Cho et al. 2011; Haque and Chand 2008). The presence of rich bioactive components of the high-protein fermented foods is paving way to gain much popularity of these foods in recent years.

Fermented Soybean Foods and Their Benefits

Fermented soybean foods are becoming increasingly important as protein-rich agricultural food products during the past several decades, with a proportional rise in their annual production (Liu 2000). Traditional soybean foods are being consumed in Asia since many centuries. Figure 11.1 shows the examples of traditional and new-generation soybean fermented food items (Kiers 2001).

Apart from preservation, fermented soybean food includes major benefits such as improved sensory quality and increased nutritional value. Fermentation imparts characteristic aroma and flavor to the fermented soybean foods (Steinkraus 1996). In few other foods, the texture is changed dramatically during fungal fermentation, thus forming a cake-like product tasting like meat. In addition, raw soybeans have been reported to contain high levels of bioactive molecules called antinutritional factors (ANFs) such as phytates. These bioactive molecules promote potential health benefits. However, majority of ANFs in soybeans are either destroyed or removed during soaking, cooking, and fermentation processes (Nout and Rombouts 1990; Tawali et al. 1998). Soybean fermentation has also been reported to improve the bioavailability of dietary iron and zinc (Hirabayashi et al. 1998; Kasaoka et al. 1997) and increase the vitamin levels in body (Denter et al. 1998; Sarkar et al. 1998).

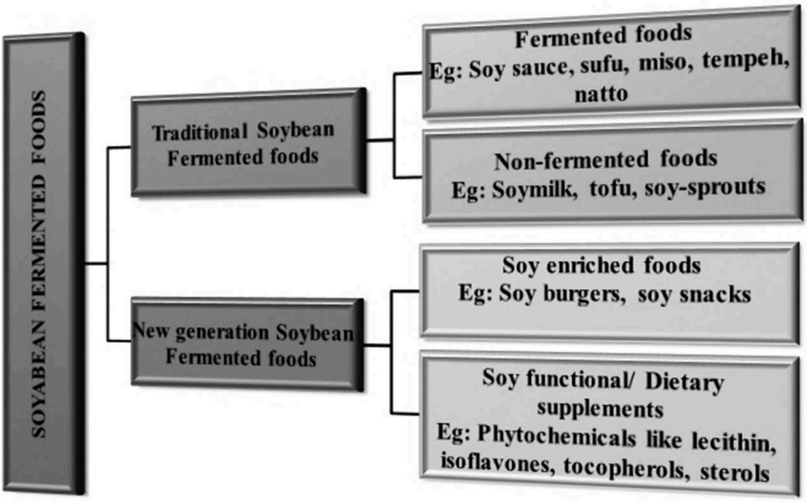
SOYABEAN FERMENTED FOODS
Traditional Soybean Fermented foods
Fermented foods
Eg: Soy sauce, sufu, miso, tempeh, natto
Non-fermented foods
Eg: Soymilk, tofu, soy-sprouts
New generation Soybean Fermented foods
Soy enriched foods
Eg: Soy burgers, soy snacks
Soy functional/ Dietary supplements
Eg: Phytochemicals like lecithin, isoflavones, tocopherols, sterols

FIGURE 11.1 Types of fermented soybean foods.

Many studies on functional peptides and proteins present in fermented food products were reported to exhibit various biological functions beyond their nutritional role (FitzGerald et al. 2004; Hartmann and Meisel 2007; Haque and Chand 2008). Their biological role includes enzymatic action (fibrinolytic) (Mine et al. 2005) and bacteriocin production by starter cultures (Leelavatcharamas et al. 2011) or food protein hydrolysis (Hartmann and Meisel 2007). The fibrinolytic activity in the fermented soybean food '*natto*' refers to the enzyme '*nattokinase*' produced by *Bacillus subtilis* subsp. Earlier researchers also reported on two other bacteria, namely, *B. amyloliquefaciens* and *B. subtilis*, isolated from fermented soybean products showing fibrinolytic activity (Peng et al. 2003; Zeng et al. 2013). The widely accepted fermented soybean foods of different origins include hawaizar (Indian), chungkookjang (Korean), douche (Chinese),

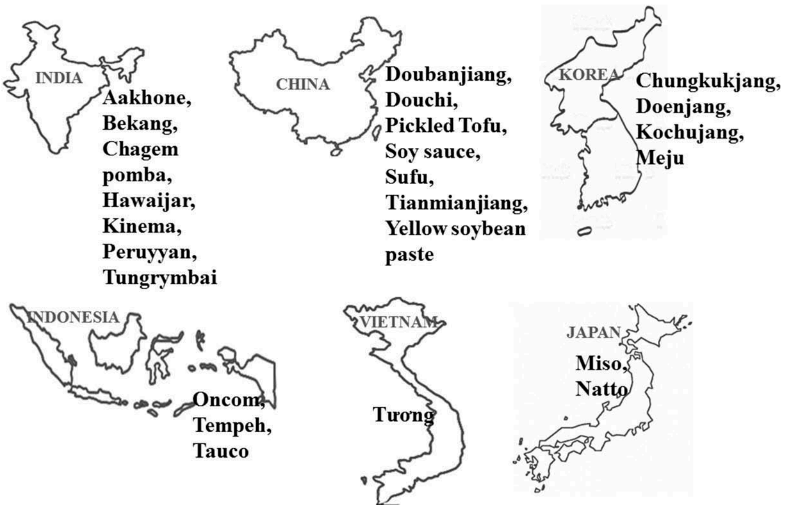

FIGURE 11.2 Fermented soybean foods of different countries.

and tempeh (Indonesian) (Mine et al. 2005; Singh et al. 2014). As reported in many studies, fermented soybeans are considered as rich source of fibrinolytic enzymes (Kotb 2012).

The present chapter focuses on different fermented soybean food products reported to be consumed in various parts of the world; it is written on the basis of various scientific reports published in both national and international sources. Figure 11.2 shows the various fermented soybean food products consumed throughout the world.

Fermented Soybean Foods from China

Out of the total soybean production, Chinese use 58% for food thus serving a large population in the world (Liu 2000). The remaining 42% are used in processed industrial products and as animal feed. Since centuries, Chinese have a practice of using microbes to convert and preserve agricultural commodities in the form of fermented food items (Chen 1989). These foods have highly improved the dietary standards of the Chinese, thus increasing the fermented dishes worldwide. Traditional Chinese fermented foods include fermented vegetables, meat products, rice wine, soy sauce, sufu, vinegar.

Doubanjiang

It is a spicy, salty bean paste prepared from fermented soy broad beans, rice, salt, and few other spices. Doubanjiang can be prepared in both plain and spicy versions. The spicy version, la doubanjiang is prepared using red chilli peppers and is particularly used in Sichuan cuisine (Shurtleff and Aoyagi 2010). This cuisine is relatively popular all over the world because of its ancestral recipe and unique flavor.

Douchi

It is most widely used in the Chinese cuisine, to make black bean sauce. Its recipe and product are quite similar to *ogiri* and *iru*, the African fermented soy bean food (Shurtleff and Aoyagi 2011).

Pickled Tofu

It is a form of processed and preserved tofu that is used as a condiment in East Asian cuisine. The ingredients typically consist of rice wine, soybeans, salt, and vinegar or sesame oil. Pickled tofu is sold in jars containing brine solution of 1 to 2 cm thick. It is available in different flavors.

Soy Sauce

Soy sauce is a condiment of fermented paste of soybeans (boiled), roasted grains, and brine solution. It contains *A. oryzae* or *A. sojae*molds as biological components. At the end of fermentation, the obtained paste is pressed well, to discharge a liquid, called the soy sauce. The solid by-product obtained after pressing is used as animal feed. Soy sauce is a traditional cooking ingredient in Southeast and Asian cuisines. Although it is originated in China during 2nd century BCE, now it is spread throughout Asia. Its usage in Western cuisine is also quite popular in recent times.

Tianmianjiang

It is a thick Chinese sauce, which is dark brown or black in color. It is prepared from fermented yellow soybeans, wheat flour, sugar, salt, and mantou. Different types based on the different compositions, production methods, and typical regional versions of sweet bean sauces are available.

Sufu

Sufu is also known as furu, a fermented soybean product from China. It is a cheesy and creamy product with spreadable consistency and significant flavor. It is popularly consumed as a side dish with breakfast of steamed bread or rice. Sufu is prepared by fungal solid state fermentation process of tofu soybean curd that is kept for aging in brine containing salt and alcohol. The stages of the process of sufu production are depicted in Figure 11.3. Various types of sufu can be categorized on the basis of color, processing method, and flavor. Based on the type of processing method, various sufu, namely, mold fermented, naturally fermented, bacterial fermented or enzymatically ripened can be prepared (Figure 11.4). Likewise, based on the

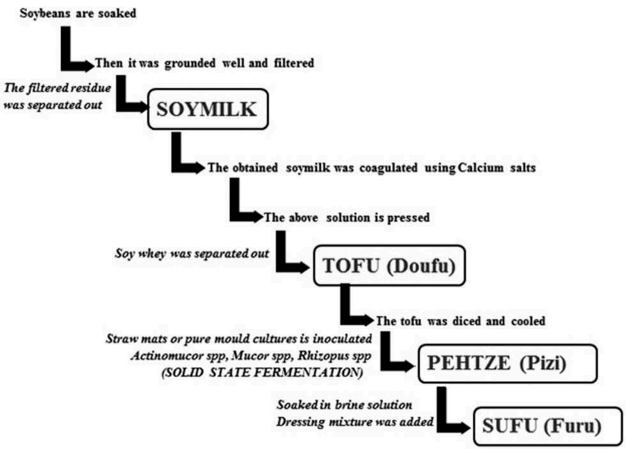
Soybeans are soaked
Then it was grounded well and filtered
The filtered residue was separated out
SOYMILK
The obtained soymilk was coagulated using Calcium salts
The above solution is pressed
Soy whey was separated out
TOFU (Doufu)
The tofu was diced and cooled
Straw mats or pure mould cultures is inoculated Actinomucor spp, Mucor spp, Rhizopus spp (SOLID STATE FERMENTATION)
PEHTZE (Pizi)
Soaked in brine solution Dressing mixture was added
SUFU (Furu)

FIGURE 11.3 Schematic representation of Sufu production.

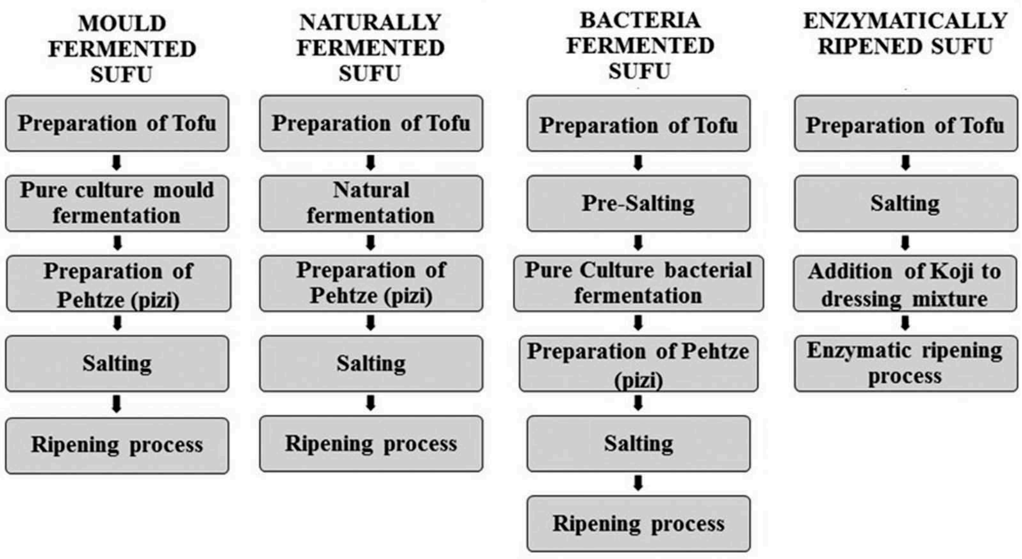

FIGURE 11.4 Different types of Sufu based on various processing technologies.

TABLE 11.1

Types of sufu based on dressing mixture

S. no.	Characteristics	Red sufu	White sufu	Gray sufu
1.	**Color**	Outside color—red to purple Inside color—light yellow to orange	Light yellow color both inside and outside	Dirty white color
2.	**Flavor and odor**	Salty	Less salty than red sufu	Strong offensive odor
3.	**Dressing mixture**	Salt, angkak (red kojic rice), alcoholic beverage, sugar, flour or soybean paste, and some spices	Similar to red sufu except angkak (red kojic rice)	Soy whey leftover from making tofu, salt, and some spices
4.	**Cuisine**	All over China	South China	Specific regions of China

color of dressing mixture, such as red, gray, and white, colored sufu can be prepared. The characteristics of red, gray, and white sufu are tabulated in Table 11.1. Various processing technologies from different regions of China are being adopted for the preparation of different types of sufu (Li 1997; Wang and Du 1998). However, it is all made using a common base, a curd obtained by adding calcium salts to soybean milk.

Yellow Soybean Paste

The ingredients of yellow soybean paste include yellow soybeans, wheat flour (additional ingredient), salt, and potassium sorbate as preservative. It is a product of China that is used often in Beijing cuisine and other northern Chinese cuisine.

Fermented Soybean Foods From India

Soybeans were brought to India from China via the Himalayas and from Indonesia via Myanmar by traders long back (Shurtleff and Aoyagi 2010). In India, some naturally fermented ethnic soybean food items include aakhone, bekang, hawaijar, kinema, peruyaan, and tungrymbai. These items are recognized as major food resources of Eastern Himalayas. This is due to the presence of highly digestive phyto-proteins with low cholesterol content, antioxidants, and other health-promoting characteristics (Tamang 2015). Despite a good demand among local people, these foods are mostly limited to household level in northeast India.

Aakhone

Aakhone is a traditional fermented soybean food from Nagaland. Its preparation is as follows: (i) soybean seeds are soaked in water and cooked well, (ii) then, the cooked beans are wrapped in fresh banana leaves and kept on the fireplace, and allowed to ferment for 5–7 d, (iii) freshly made aakhones are molded, cut into cakes, and dried in an earthen oven or dried under the sun for 2–3 d, and (iv) finally, the dried aakhones are stored in clean containers until their consumption in future (Mao and Odyuo 2007). Sometimes, pickle can also be prepared by adding green chillies, tomatoes, and salt. It can also be cooked with pork and consumed with steamed rice. The fermented aakhone can be stored for 1 week.

Bekang

It is a traditional fermented soybean food item in Mizoram and is consumed by Mizo. Its preparation begins with the collection, cleaning, and soaking of smaller-sized soybean seeds in water for ~12 h. The surplus water is drained off for boiling in an open cooker for 2–3 h or till the beans become soft. Then the cooked beans are wrapped in fresh leaves of hnahthial (*Phrynium* sp.) or nuhlhan (*Calliparpaaroria*). Then they are kept in a small bamboo basket and placed over an earthen oven or in any other warm place. The setup is left for fermentation for upto 3–4 d at ambient temperature. Bekang can be consumed as it is, or can be made as curry by adding tomatoes, green chillies, and salt. It can be consumed along with steamed rice. It is sold by Mizo women in the local shops for their livelihood (Tamang and Tamang 2009).

Chagempomba

Chagempomba is a food item in Manipur cooked with Manipuri soybean and vegetables such as spinach, mustard, and local Manipuri herbs like culantro (Awa-Phadigom), dill leaves (Pakhom), and fenugreek leaves (Methimana). People from Manipur prepare 'chagempomba' as a special food and consume with steamed rice. It can also be consumed as a condiment or as curry when cooked with other vegetables. Chagempomba is a nutritious and popular recipe among Meitei community of Manipur due to its delicious flavor.

Hawaijar

It is an ancient fermented form of soybean food consumed by Manipuris. The food is made using small soybean seeds grown locally in the hilly regions of Manipur. The preparation of Hawaijar is as follows: (i) selected soybeans are washed well and then openly boiled for 2–3 h, (ii) after boiling, remaining water is removed off and allowed to cool at 40°C, (iii) then, the cooked beans are packed well in small-sized bamboo baskets provided with lids, (iv) the baskets are lined with fresh banana or fig leaves, and (v) finally, the basket's lid is loosely sealed and kept over a warm place for upto 3–5 d for natural fermentation. The sticky texture and ammonia odor emission from the cooked soybeans assure the best quality of hawaijar. It can be stored for upto 7 d without refrigeration. There is a high demand for hawaijar among the locals in Manipur. Many women earn their livelihood by preparing hawaijar at home (Jeyaram et al. 2009).

Kinema

It is an ancient fermented soybean food item among Nepalis of Eastern Himalayas. The product is of sticky consistency and smells like ammonia (slightly alkaline), which is produced during natural fermentation (Tamang 2001).The traditional production process is as follows: (i) the selected small–sized dried soybean seeds (yellow cultivar) are washed and soaked in water overnight or for ~10 h, (ii) the soaked seeds are boiled for 2–3 h in a container with fresh water, (iii) excess water is removed and the soybean seeds are cooked and cracked softly using 'Okhli' and 'Muslo' (wooden mortar and pestle). This cracking technique is done only during kinema production to enlarge the surface area for rapid fermentation by aerobic (spore-forming) organism, *Bacillus* sp. 1% firewood ash is used to sustain the alkalinity of the fermented product, (iv) later, the soybean grits are placed inside the bamboo basket with fresh fern leaves lining, and (v) finally, the baskets are wrapped with jute cloth and allowed to naturally ferment above an earthen oven for 1–2 d (during summer) and 2–3 d (during winter).

In eastern Nepal, kinema is mostly produced by using dark brown locally grown soybean seeds than the yellow-colored seeds (Nikkuni 1997). Like ferns, banana and fig leaves are also used for wrapping. Kinema can be stored for upto 2–3 d (during summer) and 7 d (during winter) without refrigeration. It can also be preserved in dried form for several months at room temperature. Kinema curry can be consumed with steamed rice. The recipe for fresh kinema curry is to fry the onions, tomatoes, and green chillies in vegetable oil for 3–5 min. Then add turmeric powder, salt, and little water and cook in simmer for 5–7 min until the gravy is thick. Owing to its typical flavor and mucilaginous texture, kinema is liked by some people but few dislike for the same characteristics (Yamaguchi 1987).

Peruyyan

It is a traditional fermented form of soybean food of Arunachal Pradesh. In a typical peruyyan preparation, soybean seeds are washed and boiled upto 2–3 h till they become soft. The excess water is removed and placed in a bamboo basket lined with fresh ‘takiyannii’ (ginger) leaves and left for fermentation in wooden racks for 3–5 d above the fireplace. The sticky nature of the product confirms the end of the fermentation. Apatani tribes consume peruyaan along with steamed rice. It can also be consumed directly by adding hot water, chillies (tero), and salt without cooking or frying (Tamang et al. 2009).

Tungrymbai

It is a traditional fermented form of soybean food of Garo and Khasi community of Meghalaya. Its preparation is as follows: (i) selective seeds are washed well and soaked in water for 4–6 h and cooked for 1–2 h or until entire water is evaporated, (ii) then, the cooked soybeans are cooled and sealed with locally grown *Clinogynedichotoma*leaves, (iii) later, the beans are wrapped in a thick cloth and placed in bamboo basket, (iv) the covered basket is allowed to naturally ferment for 3–5 d by keeping over the fireplace. A thick curry is prepared by mixing fried onions, grinded black sesame chillies, ginger, garlic, and salt, and can be consumed with cooked rice. Tungrymbai is sold by Khasi women in the local Shillong shops (Agrahar-Murungkar 2006). People from Meghalaya also make pickle from tungrymba.

Fermented Soybean Products from Indonesia

Oncom

Oncom is a fermented cake product made of peanut (groundnut) press cake as the major ingredient, with solid waste of tapioca and soybean, coconut can be added occasionally. There are two types of oncoms available according to their mold culture; red oncom (*oncommerah)* with the main mold belongs to *Neurospora* sp. (e.g., *Neurosporacrassa, Neurosporaintermedia*, and *Neurosporasitophila).* Oncom is the only known food that contains *Neurospora* and black oncom (*oncomhitam*) with mold, *Rhizopus* sp. (e.g., *Rhizopusoligosporus)*. *Mucor* was also said to be involved in oncom fermentation. Although oncom emphasizes on difference in mold culture, some sources report that red oncom actually contains only soy pulp, whereas black oncom contains peanut press cake. Oncom is recognized as a unique food item due to its utilization of high nutritional value by-products as raw materials. The peanut press cake is obtained after the extraction of peanut oil. Similarly, the solid waste of tapioca is obtained from cassava starch extraction, soybean solid waste (soy pulp) is obtained from soymilk, and the coconut press cake is obtained from coconut milk extraction. Owing to the easy availability and cost factor of the ingredients, oncom is quite affordable to middle-low income people too. However, black oncom is usually of higher price (since some people regard it to be tastier) with smoother texture.

Oncom is usually consumed as deep-fried, stir-fried, soups, and as a filling of *combro* (fried grounded cassava) by adding *sambal*, a unique flavored seasoning. Oncom can also be roasted, seasoned with salt and sugar before eating and covered with ginger sauce. The flavor of oncom can vary as almond like, fruity, and sometimes alcoholic. When it is fried, it imparts minced meat flavor. The alcoholic flavor is lost after cooking because of heat degradation. Oncom bandung is considered as best oncom in Indonesia, as raw peanuts are used as the main ingredients instead of peanut press cake.

Benefits of Oncom

- It contains high protein (black oncom) and less fat content.
- It is good diet-conscious food.
- It improves nutrient absorption.
- It contains lot of fungal enzymes that help in carbohydrate, protein, and fat catabolism.
- It also prevents flatulence due to oligosaccharides content of peanut and soybean.

The biggest concern of oncom is the presence of aflatoxin, the peanut toxin that can cause liver cancer, if accumulated. However, the amount of aflatoxin decreases up to 50% after fermentation. Moreover, phytic acid in peanut acts as antinutritional content that inhibits absorption of minerals, namely, calcium and magnesium. There was a research report that oncom could reduce the cholesterol levels in rats, so it might reduce cholesterol level in humans too (Beuchat 1976).

Tempeh

Tempeh is a popular food product in Indonesia prepared from fermented soybean. It is prepared naturally through controlled fermentation process that includes addition of a tempeh starter, which is a mixture of live fungus. It becomes like cake after 1 or 2 d.

Benefits of Tempeh

- Tempeh lowers/maintains cholesterol (due to the presence of niacin in tempeh).
- It boosts bone health (due to high calcium and copper content in tempeh).
- Maintains blood sugar in the body.
- Possesses anticancer properties.
- It increases the number of beneficial bacteria in the gut.
- Tempeh can also improve the immune system in the body.
- Moreover, presence of copper in tempeh plays an important role in bone growth.
- It has antiinflammatory properties (due to isoflavones in tempeh).
- Antioxidants in tempeh play a central role in controlling heart disease, diabetes, and cancer.

Tauco

Tauco is a common soya paste in Indonesia prepared from preserved fermented yellow soybeans. It is made by boiling yellow soybeans, then grinding and mixing them with flour. After this, the mixture is subjected to fermentation to make a soy paste and soaked in salt water followed by drying in the sun for several weeks till the color of the paste turns yellowish-red. A good quality tauco has distinct flavor and is generally used in cooking by Chinese Malaysian.

Fermented Soybean Foods from Japan

Miso

Miso is a common fermented food item in Japan prepared from soybean, which is made by grinding and mixing steamed soybeans with koji and salt. The mixture is then allowed to ferment for a period of 4 to 12 months. Traditional Japanese miso is prepared from soybean, which is gluten-free. Cultured millet or wheat or combinations of different grains and beans are used for

TABLE 11.2

Comparative characteristics of different types of Miso

S. No.	Characteristics	White miso	Red miso	Yellow miso	Barley miso
1.	**Ingredients**	Prepared from soybeans and rice	Prepared from high proportion of soybeans	Prepared after fermenting the white miso	Prepared from soybeans and barley
2.	**Fermentation time**	1 week and 2 years (called mame)	3 years	Slightly longer period than red miso	Longer period than white miso
3.	**Color**	White and dark brown (mame)	Russet colored	Yellow colored	Whitish gray
4.	**Flavor and taste**	Sweet to mild salty	Deep flavor and salty		Strong barley aroma, sweet in taste

the preparation of miso pastes. The duration of fermentation period sometimes affect the flavor. The texture of different types of miso varies and the taste also varies from sweet to mild salty. The taste of miso is more salty when it is prepared from whole grain of soybean instead of hulled grain.

Types of Miso

Based on different ingredients, there are different types of miso (Table 11.2), (1) white miso—shiro, (2) red miso—aka, (3) yellow miso—shinso, and (4) barley miso—mugi. It can be added to all types of dishes including soups/broths, stews, salad dressings, glazed vegetables, and marinades.

Nutritional Benefits of Miso

- Miso is rich in protein and essential minerals.
- It is a good source of vitamins B, E, K, and folic acid.
- Miso promotes human gut beneficial bacteria.
- Since miso has high salt content and hence should be consumed within the permissible limit of not more than 6 g/day.

Natto

Natto is a traditional Japanese food item prepared from boiled soybeans with ‘natto’ yeasts grown on the surface. It has a special flavor. When natto yeasts grow, they make it nutritious by dissolving the proteins in soybeans for easy absorption and release various enzymes and vitamins. Natto contains a good amount of fiber which supports digestive health. Natto moves throughout the body in undigested form and adds bulk to stool to alleviate constipation. It contains high levels of vitamin K, which is involved in calcium metabolism and maintains bone health. Natto is slippery in texture and has very strong aroma. The fermentation process of natto is short compared to miso and it takes 20–22 h from the beginning. It takes about 3 d from the start of processing to shipment.

Preparation of Natto

Generally small-sized soybean seeds are preferred for the preparation of natto because small beans can be mingled easily with rice and it becomes pleasant in taste. In addition, smaller soybeans have larger surface area, which in turn produces more sticky paste and delicious flavor of natto, creating better taste. The major steps involved in the preparation of natto are as follows (Cai 2002). (i) Soybeans are selectively chosen and washed in bean-washing machine to remove their skin, (ii) soybeans are soaked in enough water (shineski process) for 17–18 h (in winter) and 7–8 h (in summer). Soaking time differs according to the size of the soybean and temperature of the water, (iii) soybeans are cooked in pressure cooker, as the steaming process kills the bacteria on the soybeans surface. In addition, soybeans become soft and natto yeasts can take nutrition and grow easily. Moreover, steaming causes the release of various enzymes produced by natto yeasts and it becomes easy to penetrate and dissolve the ingredients, (iv) after steaming, purely cultured natto yeasts are sprayed at 70–90 °C on the steamed soybeans so that the yeasts adhere on their surface to prevent bacterial contamination, (v) the boiled beans are packed in the container in paper cups, plastic bags, styrofoam and so on. As natto yeasts need oxygen for their growth, soybeans should not be packed too tightly, (vi) soybeans are kept in fermentation room for fermentation at 38–42 °C for 16–24 h. At the end of 16–24 h, all the products are taken out of the room and transferred to refrigerating room for cooling, to stop the fermentation process, (vii) maturation is a process of refrigerating the natto yeasts at 5 °C or below. Sufficient care is taken to prevent the rise of product temperature during wrapping, to control natto yeasts growth. Control of temperature from fermentation to maturation is very important for successful preparation of natto, (viii) finally, the products are wrapped in containers, and stored in refrigerator till shipment.

Health Benefits

- Natto is locked with several nutrients making them a super nutritious fermented food.
- It improves digestion process.
- It helps in stronger bones.
- It strengthens the immune system of human body.
- It plays an important role in promoting heart health.

Fermented Soybean Foods from Korea

Fermented soybean products such as chungkukjang, kanjang (soy sauce), doenjang, and kochujang are well accepted by the people of Korea. Chungkukjang is a soybean product, which is fermented for a short period like Japanese food ‘natto’. Other items, namely, kanjang (soy sauce), doenjang, and kochujang need fermentation for longer period. Chungkukjang is usually prepared during autumn and winter (soybean harvest period). Traditionally, it is prepared by fermenting the noncrushed cooked soybeans for 2–3 d in the living room. Doenjang and kanjang are prepared as follows. In the first step, dried soybean chunk is prepared from cooked soybeans (meju) in late October or early November. Meju is allowed to ferment for 1–2 months below the outdoor roof of the Korean traditional houses during winter. In the second step, meju is aged for 1–2 months in large earthenware jars containing salt solution for liquid fermentation. The supernatant liquid is decanted to prepare kanjang and the remaining soy paste is used to prepare doenjang. At last, both paste and the liquid are left for aging for longer period of time. Kochujang is considered as a unique Korean traditional food for many years (Kwon et al. 2011). Various health benefits of Korean food products are tabulated in Table 11.3. Preparation of various Korean food products from fermented soybean has been presented in Figure 11.5.

TABLE 11.3

Health benefits of Korean soybean fermented foods

Soybean fermented food	Health benefits	References
Chungkukjang	Lowers blood pressure and serum lipids	Yang et al. 2003
	Exerts hypoglycemic effects along with antioxidant effects in diabetic rats	(Kwon et al. 2007)
	Possesses antidiabetic activity (due to the presence of isoflavonoid aglycones)	Jang et al. 2006; Kim 1999; Kwon et al. 2007; 2010
	Extracts of chungkukjang possess highest degree of free-radical-scavenging capacity and tyrosinase inhibition	Choi et al. 2008
	Prevents hyperpigmentation	
	Has antiobesity effects	Anderson and Pasupuleti 2008
Doenjang	Has antimetastatic, anticancer, antihypertensive, and antimutagenic properties	Park et al. 2003; Jung et al. 2006; Yang et al. 2009; Butler et al. 2003
Kochujang	Has antimutagenic activity and antitumor effects	Kim et al. 1999; Park et al. 2001;
	Has antiobesity properties	Ashiuchi et al. 2001; Choo 2000;
	Reduces fat deposition and decreases circulation level of cholesterol, triglycerides and blood pressure	Koo et al. 2008

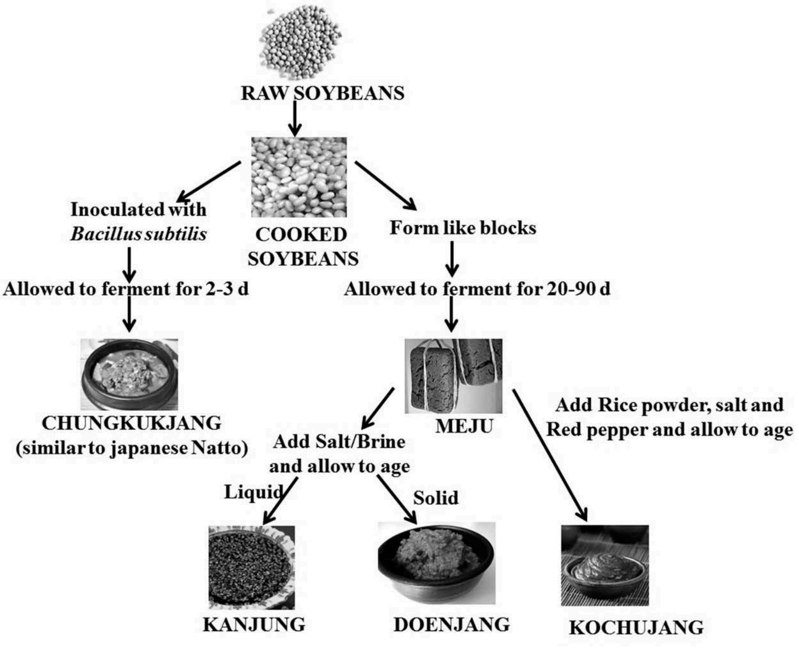
RAW SOYBEANS
Inoculated with *Bacillus subtilis*
COOKED SOYBEANS
Form like blocks
Allowed to ferment for 2-3 d
Allowed to ferment for 20-90 d
CHUNGKUKJANG (similar to japanese Natto)
MEJU
Add Salt/Brine and allow to age
Add Rice powder, salt and Red pepper and allow to age
Liquid
Solid
KANJUNG
DOENJANG
KOCHUJANG

FIGURE 11.5 Schematic representation of preparation of different Korean soybean fermented products.

Fermented Soybean Foods from Vietnam

Tương

Tương is a fermented form of soybean product commonly used in Vietnamese cuisine. It is prepared in the form of thick paste or thin liquid. Salty paste of *tương* is popular in vegetarian meals and is highly preferred by Vietnamese Buddhist monks. The dark brown color paste of *tương* is produced through addition of *A. oryzae* (a type of fungi) to the roasted soybeans. Later, it is allowed to naturally ferment in a jar containing water till the development of umami flavor. Other ingredients, namely, maize powder/glutinous rice along with salt or water, can be added too.

Different varieties of *tương* are prepared in various parts of the country. The varieties that are made in Central Vietnam are of watery consistency, with solids separating out at the bottom of the fermentation jars. On contrary, a thick or condensed form known as *tươnglàngBần*or *tươngBần* is prepared in a town, BầnYênNhân, in northern Vietnam near Red River Delta region and in MỹHào district of HưngYên Province. Few other forms of *tương* are similarly named after their districts or towns from where they are prepared. They include, *tươngPhốHiến* (prepared in HưngYên Province), *tương Nam Đàn* (prepared in Nghệ An Province), *tươngCựĐà* (prepared in HàTây Province), and *tươngchùaMía* (prepared in ĐườngLâm village of HàTây Province). Soy sauce is referred as ‘*nước tương*’ in southern Vietnam.

Conclusion

Besides meat, providing a good and cheap protein source to the global population is a big challenge throughout the world. Alternative protein sources include cereals and legumes. Fermentation can improve digestibility and add desirable flavors, which is especially true in case of fermented form of soybean products. These fermented foods are very much acceptable in terms of quality, nutritional properties, and functionalities. Moreover, their industrial manufacture can be an added advantage for developing economies. Fermented soybean food products are well known for the prevention of heart disease, breast cancer, and obesity. But the scientific/research reports on these beneficial health aspects are not well documented. This urges a need to educate the people regarding the beneficial effects of fermented soy products. So, in the present chapter, we have compiled the detailed information on various fermented soybean products which are being consumed by the people of many countries throughout the world as sources of protein-rich diet.

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12

Nutritional and Health Benefits of Idli *and* Dosa

Srinivasan Ramalingam
Department of Food Science and Technology, Yeungnam University, Gyeongsan, Republic of Korea

Sujatha Kandasamy
Department of Food Science and Technology, Pondicherry University, Pondicherry, India

Ashutosh Bahuguna and Myunghee Kim
Department of Food Science and Technology, Yeungnam University, Gyeongsan, Republic of Korea

1.1 Introduction

Fermented foods are an important part of everyday human diet in many cultures around the globe due to their high nutritional quality, health benefits, characteristics, organoleptic properties, short preparation period, sustained shelf life, aroma, and flavor (Reddy *et al.*, 1982; Das and Deka, 2012; Kandasamy *et al.*, 2018). The fermented foods are classified according to the chief ingredients of the fermented product. Food materials such as legumes, cereals, vegetables, fruits, starchy tubers, meat, milk, and fish are some common raw materials used in the preparation of fermented foods (Scott and Sullivan, 2008; Kandasamy *et al.*, 2018). Cereals and pulses are a major nutritional source for humans, which provides 50% of the daily calorie requirement, and these are primary dietary components of most the developing countries in Asia and Africa.

Digestibility of foods prepared from the conventional cereals and legumes is relatively lower than the foods prepared from animal sources such as meat and fish. The presence of a high quantity of insoluble fibers, complex proteins, and anti-nutritional factors in the plant-based foods reduces its digestion ability leading to less nutritive value. People of African and Asian countries convert these difficult-to-digest cereals and legumes into nutrient-rich food via fermentation (Nout, 2009). Since ancient times, a significant portion of cereals and pulses in the human diet has been utilized through the fermentation process. Thus, many cultures adopted a number of indigenous fermentation methods to prepare a variety of cereals and pulses based on fermented foods (Blandino *et al.*, 2003). Among the number of the fermentation process, cereal-based fermented food prepared by fortification of legumes is a common one. The food prepared with a combination of cereal-legume is nutritionally advantageous as it provides high carbohydrates from cereals and proteins from legumes (Blandino *et al.*, 2003). Cereal-legume-based fermented foods are predominantly consumed in South and far East Asian countries and few countries of South America and Africa (Mehta *et al.*, 2012).

Fermented cereal- and legume-based products are currently being promoted in developing countries due to their purported health benefits. Several fermented foods in India are regionally specific, homemade, and prepared using unique traditional methods (Das and Deka, 2012). The raw material availability, varied climatic conditions, and diverse cultural practices in India have resulted in numerous traditional fermented food recipes using cereals and legumes (Sekar and Mariappan,

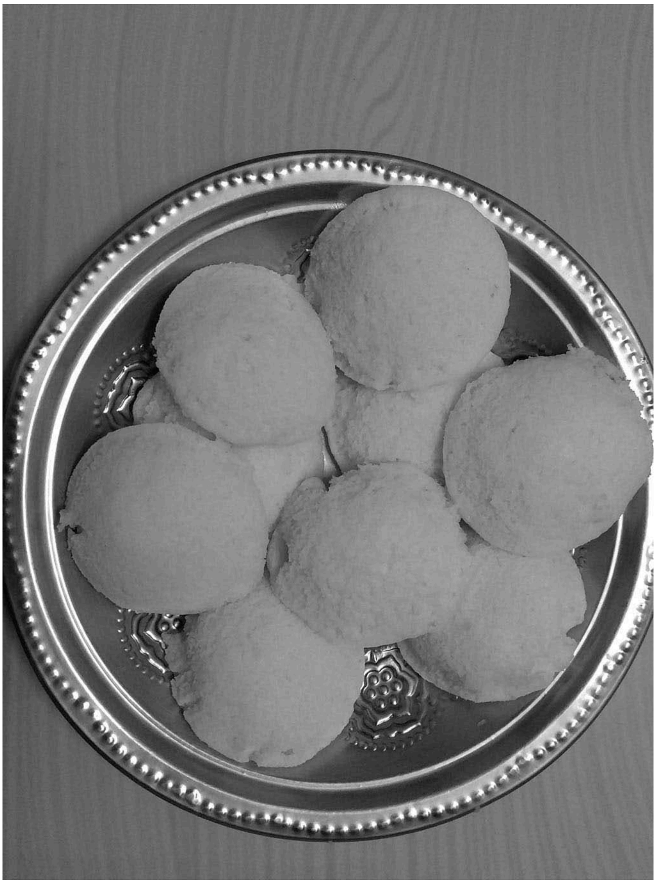

FIGURE 12.1 Steamed *idli*.

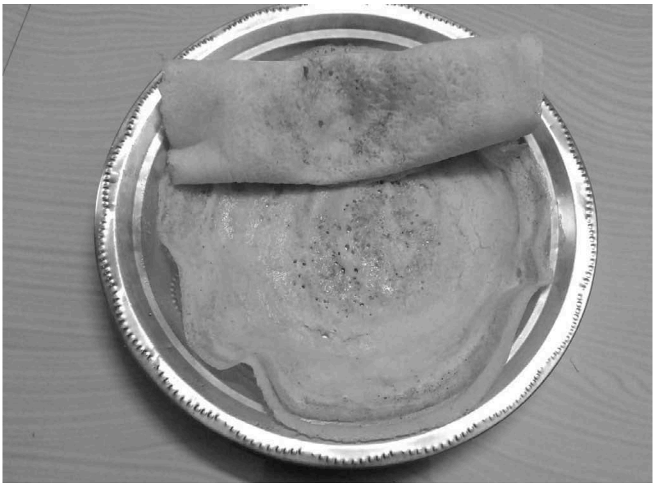

FIGURE 12.2 Roasted *dosa*.

2007). Among the several Indian traditional foods, *idli* (Figure 12.1) and *dosa* (Figure 12.2) are cereal- and legume-based indigenous food prominent in South India and Sri Lanka consumed as breakfast, dinner, and/or snack, which are served with *sambhar* (lentil-based vegetable stew) and coconut *chutney* (Reddy *et al.*, 1982; Sridevi *et al.*, 2010). On average, South Indians consume *idli* and *dosa* three to four times a week. Large scale production and consumption of *idli* and *dosa* were observed in Indian restaurants and industrial canteens (Nagaraju and Manohar, 2000).

Idli and *dosa* are prepared from natural fermented thick (semisolid) batter ground from dehulled black gram (*Phaseolus mungo* L., synonym name *Vigna mungo*) and polished white rice (*Oryza sativa* L.). *Idli* is a white-colored, round-shaped, acid-leavened, soft, spongy (porous in texture), steam-cooked pancake with characteristic desirable sour flavor (Blandino *et al.*, 2003; Shetty *et al.*, 2005) similar to steamed sourdough bread in Western countries (Nisha *et al.*, 2005). *Dosa* is a thin, fairly crisp, fried, pancake-like food (Soni *et al.*, 1985). *Idli* and *dosa* act as a desirable dietary food for people of all ages, from infants and grown-ups to invalids, because the foods prepared from such batters are easily digestible and possess high nutritional value, which imparts its popularity and rising demand not only in India but also across countries. In recent times, commercial production of ready-to-cook *idli* and *dosa* batter and ready-mix is an emerging business in India and these are exported to several countries of the Gulf, Asia, and Europe. Also, special varieties of fluffy *idli* are exported in hundreds of kilos every day to Malaysia and Singapore restaurants from India.

1.2 History

Idlis and steaming process were common in India from early 700 CE. The steaming process was possibly introduced from Indonesia approximately between 800 and 1200 CE. *Idli* is consumed in South India during 1100 CE, and the honor of bringing them into the nation's regular diet duly goes to the South Indian housewives, predominantly Tamil Nadu. Between 1485 and 1600 CE, the *idli* has been related to the moon in literature. As per a literature finding during 920 AD, the term "*idli*" is indicated in the Kannada writing of Shivakotiacharya. As stated by the poet Chavundaraya, during 1025 AD, *idli* is made by using a grounded paste of black gram soaked in buttermilk, combined with asafoetida, cumin, coriander, pepper, and clear water of curds before shaping. In the Sanskrit book *Manasollasa*, written by Western Chalukya King Someshvara III during 1051 AD, *idli* is prepared using fine urad flour, shaped into small balls, fried in ghee, and seasoned by asafoetida, cumin, and pepper. *Dosa* is also described in Tamil Sangam literature in about the sixth century AD (Ramakrishnan, 1979; Chavan and Kadam, 1989; Purushothaman *et al.*, 1993).

1.3 Basic Ingredients and Preparation of *Idli* and *Dosa*

Rice, black gram, salt, and water are the main constituents for the preparation of *idli* and *dosa* batter (Figure 12.3). The quality of *idli* and *dosa* mainly depends upon the choice of variety and quantity of rice and black gram. The rice cultivars and variety used along with the difference in fermentation conditions is highly affecting the sensory profile of *idli* and *dosa* (Kumar *et al.*, 2005). *Idli* and *dosa* are commonly prepared using dehulled black gram along with polished white rice and/or rice grits. The selection of rice or rice grits such as parboiled rice, raw rice, and a mixture of both depends on the consumer's choice. Black gram in the *idli* and *dosa* batter has a vital role in the formation of the foamy texture of the *idli* and *dosa* because of many surface-active materials, such as globulin proteins and arabinogalactan, which retain the gases produced during the fermentation process (Reddy *et al.*, 1982; Nisha *et al.*, 2005). The ratio of rice to the black gram in *idli* batter preparation varies from 2:1 to 4:1 (w/w). However, a higher proportion of rice is not preferable due to the dominance of rice flavor (Steinkraus, 1996). Hence, rice and black gram in the ratio of 2:1 or 3:1 is suggested by most of the literature to prepare *idli* with superior quality and sensory traits (Sridevi *et al.*, 2010; Ghosh and Chattopadhyay, 2011). *Dosa*

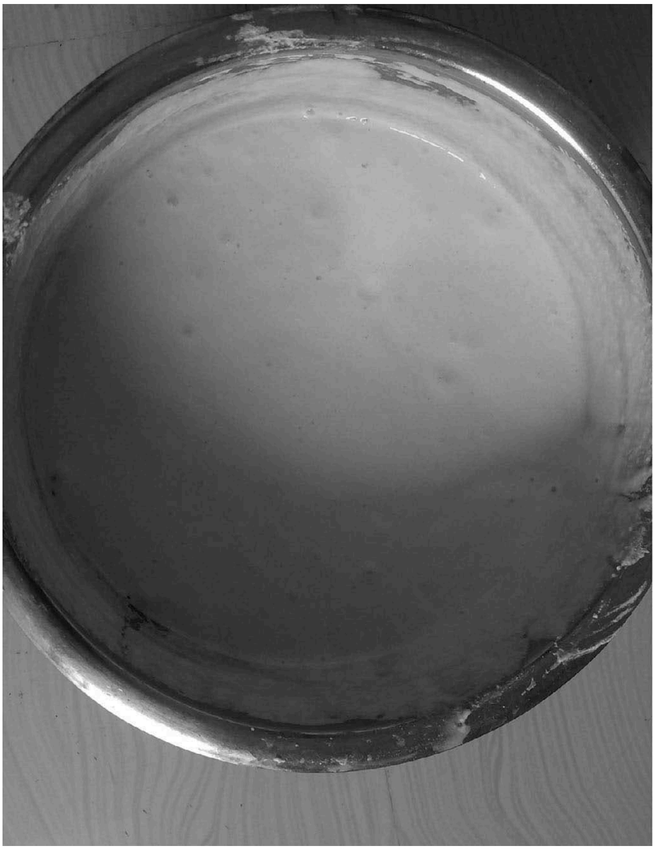

FIGURE 12.3 Fermented *idli* and *dosa* batter.

batter is similar to *idli* batter, but the batter is thinner in consistency (Soni *et al.*, 1985; Steinkraus, 1996). However, the addition of raw rice with parboiled rice in 1:3 (w/w) ratio along with one part of black gram is preferable to attain the desired crispness of *dosa*.

The conventional *idli* and *dosa* batter preparation process is preceded by three major steps—soaking of raw materials, wet grounding of raw materials, and the fermentation process (Reddy *et al.*, 1982). The series of steps in the preparation of *idli* and *dosa* batter is depicted in Figure 12.4. Initially, black gram and rice are washed and soaked individually in double the volume of water for about 5–10 hours or overnight. The soaked black gram and rice are washed and grounded separately using a wet grinder or mill with a little addition of water. Next, the coarsely grounded rice slurry is mixed with finely grounded black gram along with required salt and water. Salt is also an essential component in fermentation which improves the taste of *idli* and *dosa* making it highly palatable (Soni and Sandhu, 1989). In the literatures, 0.85–2% (w/v) salt has been utilized for effective fermentation. To make a batter of a desirable and uniform consistency, the required amount of water varies from 1:1 to 1:5 times dry weight (w/w) of the batter ingredients (Reddy *et al.*, 1982). The batter is then allowed for natural fermentation in a warm place (30°C) for 12–18 hours/overnight or till the original volume increases about 2.5 times. A closed vessel is used for batter fermentation in order to prevent contamination and to keep away from insects (Nisha *et al.*, 2005; Rao *et al.*, 2005).

Supplementation of fenugreek seeds (5–10 g) and rice flakes (30–40 g) along with black gram and rice in preparation of *idli* and *dosa* batter is also observed in South India, predominantly Tamil Nadu. These additional ingredients are believed to improve the consistency of the batter,

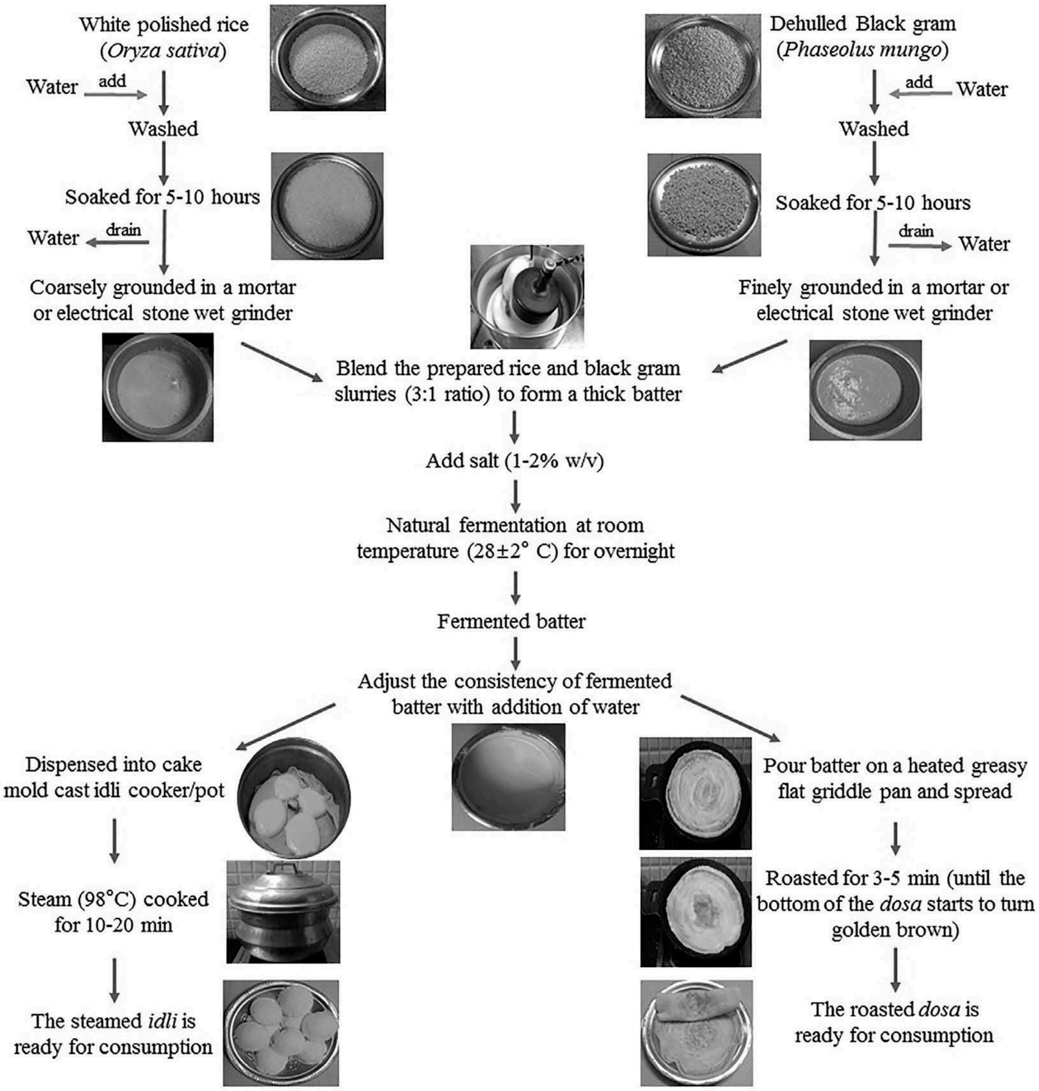
White polished rice
(*Oryza sativa*)
Water
add
Washed
Soaked for 5-10 hours
Water
drain
Coarsely grounded in a mortar or electrical stone wet grinder
Dehulled Black gram
(*Phaseolus mungo*)
add
Water
Washed
Soaked for 5-10 hours
drain
Water
Finely grounded in a mortar or electrical stone wet grinder
Blend the prepared rice and black gram slurries (3:1 ratio) to form a thick batter
Add salt (1-2% w/v)
Natural fermentation at room temperature (28±2° C) for overnight
Fermented batter
Adjust the consistency of fermented batter with addition of water
Dispensed into cake mold cast idli cooker/pot
Steam (98°C) cooked for 10-20 min
The steamed *idli* is ready for consumption
Pour batter on a heated greasy flat griddle pan and spread
Roasted for 3-5 min (until the bottom of the *dosa* starts to turn golden brown)
The roasted *dosa* is ready for consumption

FIGURE 12.4 Schematic representation of the preparation of *idli* and *dosa*.

thereby improving the texture and flavor of *idli* and *dosa*. Literatures revealed that several attempts have been made to replace the conventional black gram and rice in *idli* and *dosa* batter with other legumes including soybean (*Glycine max*), chickpea, green gram, common beans (*Phaseolus* Sp.), mungbean (*Phaseolus radiates*), horse gram (*Macrotyloma uniflorum* L.), and Great Northern beans (*Phaseolus vulgaris* L.); cereals including bhagar (common millet, *Panicum miliaceum*), brown rice, amaranth grains, foxtail millet, finger millet (*Eleusine coracana*), and pearl millet (*Pennisetum glaucum*); and additives such as leaves of *Murraya koenigii* and *Moringa oleifera* for the preparation of certain novel *idli* and *dosa*-like products (Rekha and Vijayalakshmi, 2011; Palanisamy *et al.*, 2012; Uma Maheswari and Shetty, 2013; Manickavasagan *et al.*, 2013; Nazni and Durgadevi, 2014; Narayanan *et al.*, 2016; Chelliah *et al.*, 2016). Even though the substitutive ingredients could add more nutritive value to *idli* and *dosa*, the acceptability of these products among consumers is little and acceptability depends upon the organoleptic properties and final texture and flavor.

To make *idli*, a special pot containing a stack of the pan with smooth and round indentations for placing *idli* batter is employed. The perforated pans are kept above the level of boiling water in the steaming pot for allowing the steam to rise and to evenly cook *idli*. Depending upon the pot size, the steaming time varies from 10–25 minutes. *Dosa* is prepared by pouring a ladle full fermented *dosa* batter on a heated greasy flat griddle pan, spread using a ladle (backside), and roasted (until the bottom of the *dosa* starts to turn golden brown). *Dosa* texture is measured in terms of eye formation (small apertures on *dosa*) (Soni *et al.*, 1985). In olden times, while *idli* pan was not commonly available the batter is poured onto a cloth firmly fixed onto the mouth of a bowl-shaped, deep cooking pan partly filled with water. The pan is closed using a heavy lid and kept on heat till the *idli* was cooked. Based on the border of the pan, *idli* is normally large which is often cut into bits and dished with *sambhar* and/or coconut *chutney* (Soni and Sandhu, 1999; Soni and Marwaha 2003; Panesar and Marwaha, 2013). Based on raw materials a variety of *idlis* such as *Malligae idli*, *Kanchivaram idli*, *Rava idli,* and *Soy idli* are available currently.

1.4 Microflora Involved in the Fermentation of *Idli* and *Dosa* Batter

Idli continues to be extensively widespread in India due to its distinguishing consistency and flavor change that occurs during fermentation (Nisha *et al.*, 2005). *Idli* is used as a breakfast food in all seasons. Due to the fermentation process, *idli* is easily digestible. Traditionally, *idli* has been prepared through a natural fermentation process (Mukherjee *et al.*, 1965). In general, cereal fermentations are carried out by the lactic acid bacteria (LAB), yeasts, and molds. The microbial fermentation plays an important role in the development of desirable texture, flavor, and enhancement of product shelf life along with the enrichment of nutrition (Steinkraus, 1996). The natural biota involved in fermentation is highly influenced by the ingredients and the environment (Desikachar *et al.*, 1960). The raw materials such as black gram and rice are the main reservoir of natural microflora which ferments the *idli* batter during the natural fermentation process (Radhakrishnamurthy *et al.*, 1961). In *idli* and *dosa* batter, rice contributed yeast and bacterial flora which produce amylases resulting in the formation of reducing sugars, whereas the bacterial flora that produce proteinases and gases are mainly contributed by the black gram (Soni *et al.*, 1986). During fermentation of *idli* batter, two noteworthy changes—acidification and a leavening of batter—occurs. The acidity of the batter and microbial population increase subsequently by a decrease in pH during fermentation. The lactic acid concentration in the batter ranged between 1.2% and 2.5% approximately with a final pH 4.5, after 12 to 18 hours of fermentation at room temperature (30°C) (Mukherjee *et al.*, 1965). Concurrently, as a result of carbon dioxide formed by the microbes the batter volume increases (Steinkraus, 1996).

The microorganisms involved in *idli* and *dosa* batter fermentation have been enumerated through the plate count method and identified using microscopic and biochemical methods (Soni *et al.*, 1986). For total plate counts of LAB, MRSA (De Man, Rogosa, and Sharpe Agar) and for yeast, potato dextrose agar (Rekha and Vijayalakshmi, 2011) and Sabouraud dextrose agar (Sridevi *et al.*, 2010) have been employed. Moreover, for counting aerobic or mesophilic organisms tryptone glucose yeast extract agar (Mukherjee *et al.*, 1965) and nutrient agar (Rekha and Vijayalakshmi, 2011) were used. A limited number of modern molecular identification and typing studies are carried for better identification of the microbes involved in the *idli* batter fermentation, but each stage of batter fermentation is not yet studied with the modern techniques (Agaliya and Jeevaratnam, 2013).

Idli fermentation is obviously governed by native LAB and yeast, which develops while soaking of rice and black gram (Mukherjee *et al.*, 1965). Each ingredient independently contributes to a diverse variety of indigenous LAB and yeast. Dynamics in microbial population during fermentation is also influenced by the alteration in temperature and climatic conditions. For instance, LAB and yeast greatly dominate during winter, although in summer yeasts decline probably due to a higher temperature (Soni *et al.*, 1986). The dominant microorganisms periodically grow one after another from soaking and continue the batter fermentation. In freshly fermented *idli* batter, LAB and yeast are in huge numbers varying from 10^9–10^{11} CFU/g (Nisha *et al.*, 2005; Sridevi *et al.*, 2010; Iyer *et al.*, 2011). Although the synergism between LAB and yeasts is not yet ascertained, Venkatasubbaiah *et al.* (1984) stated the role of LAB is to increase the acidity level in the batter by reducing the pH, thereby favoring the growth of the yeast. Consecutively, yeast balances the growth of LAB by breaking starch through extracellular amylolytic enzymes. Although previous studies (Mukherjee *et al.*, 1965; Ramakrishnan, 1979) stated that the LAB mainly *Leuconostoc mesenteroides* and *Lactobacillus fermentum* are responsible for the leavening process, later the leavening role of yeasts is also confirmed (Soni *et al.*, 1986; Sridevi *et al.*, 2010). Until now numerous species of LAB and yeast have been reported to be involved in *idli* fermentation (Table 12.1).

TABLE 12.1

Microorganisms isolated from *idli* and *dosa* batter

Organisms	References
Bacteria	
Bacillus amyloliquefaciens	Saravanan *et al.* (2015); Soni *et al.* (1986)
Bacillus cereus	Roy *et al.* (2007); Saravanan *et al.* (2015)
Bacillus polymyxa	Soni *et al.* (1986)
Bacillus subtilis	Saravanan *et al.* (2015); Soni *et al.* (1986)
Bacillus tequilensis	Saravanan *et al.* (2015)
Enterobacter sp.	Soni *et al.* (1986)
Enterococcus cloacae	Saravanan *et al.* (2015)
Enterococcus faecium	Vijayendra *et al.* (2010)
Lactobacillus acidophilus	Bhattacharya and Das (2010)
Lactobacillus delbrueckii	Soni *et al.* (1986)
Lactobacillus fermentum	Bhattacharya and Das (2010); Soni *et al.* (1986)
Lactobacillus pentosus	Agaliya and Jeevaratnam (2013)
Lactobacillus plantarum	Agaliya and Jeevaratnam (2013); Bhattacharya and Das (2010); Gupta and Tiwari (2014); Iyer *et al.* (2011)
Lactobacillus plantarum subsp. *argentoratensis*	Agaliya and Jeevaratnam (2013)
Lactococcus lactis	Iyer *et al.* (2011); Saravanan *et al.* (2015)
Leconostoc lactis	Saravanan *et al.* (2015)
Leuconostoc mesenteroides	Mukherjee *et al.* (1965); Soni *et al.* (1986)
Micrococcus luteus	Saravanan *et al.* (2015)
Micrococcus varians	Soni *et al.* (1986)
Pediococcus cerevisiae	Mukherjee *et al.* (1965); Soni *et al.* (1986)
Pediococcus pentosaceus	Saravanan *et al.* (2015)
Streptococcus faecalis	Mukherjee *et al.* (1965); Soni *et al.* (1986)
Weissella cibaria	Saravanan *et al.* (2015)
Weissella confusa	
Yeast	
Candida kefyr, *Candida krusei*, *Candida robusta*, *Debaryomyces hansenii*, *Hansenula anomala*, *Kluyveromyces*	Soni *et al.* (1986)

(*Continued*)

TABLE 12.1 (Cont.)

Organisms	References
marxianus, *Oosporidium margaritiferium*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*, *Trichosporon beigelii*	
Torulopsis candida	Reddy *et al.* (1982); Soni *et al.* (1986)
Trichosporon pullulans	Batra and Millner (1974); Soni *et al.* (1986)

Mukherjee *et al.* (1965) confirmed the major LAB involved in this process is heterofermentative *L. mesenteroides* and homofermentative *Streptococcus faecalis* which regulate the acidity of batter. Other LAB groups include *Lactobacillus fermentum*, *L. plantaraum*, *L. coryneformis*, *L. delbrueckii*, *L. lactis*, and *Pediococcus cerevisiae* which are also involved in the *idli* batter fermentation. Likewise, yeasts such as *Saccharomyces cerevisiae*, *Hansenula anomala*, *Debaryomyces hansenii*, *Torulopsis candida*, and *Trichosporon beigelii* are reported in the fermentation of *idli* batter. In addition to the above-mentioned organisms, *Weissella confusa*, *Weissella cibaria*, *Leuconostoc lactis*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Bacillus subtilus*, *B. amyloliqefaciens*, *B. tequilensis*, *B. polymyxa*, *Enterobacter sp.*, and *Micrococcus varians* have been reported from *idli* batter (Soni *et al.*, 1986; Saravanan *et al.*, 2015). Nevertheless, the existence of *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Enterococcus cloacae*, and *Staphylococcus aureus* along with natural biota was reported in *idli* batters obtained from the markets (Roy *et al.*, 2007; Saravanan *et al.*, 2015).

1.5 Storage and Preservation

Generally, *idli* batters are prepared at home and are mostly consumed within 2 to 3 days, where the batters are in a refrigerated environment. Recently, there has been a great demand in several parts of India for ready-to-cook batter with a shelf life of 4 to 5 days at 4°C (Nisha *et al.*, 2005). Increase in storage time declines the quality of *idli* batter owing to the existence of high bacterial and yeast counts (~10^{10} CFU/g) and their activity (Sridevi *et al.*, 2010). However, the literature revealed that the efforts made to improve the shelf life of *idli* batter are not enough. To address the shelf-life problem of *idli* batter few attempts were made such as canning of *idli*, use of defined starter cultures, the addition of batter stabilizers, modification of packaging atmospheric environment, and drying of *idli* batter for a ready-to-reconstitute. The fermented *idli* batter that was canned and processed at 125°C for 60 minutes (which formed the *idli*s in the can itself) possessed good organoleptic properties until 6 months at 26°C (Nagarathnamma and Siddappa, 1965). Durgadevi (2012) stated that modified atmospheric packaging (with oxygen, nitrogen, and carbon dioxide gas) of *idli* batter increases the shelf life up to sevenfold under room temperature upholding a good sensory quality.

Padmashree *et al.* (2014) prepared ready-to-reconstitute *idli* by using various drying (cabinet drying, fluidized bed drying, and microwave drying) technologies and stored 37°C. However, these drying methods have significantly affected the chemical stability, organoleptic properties, color, and texture of the *idli*. Nisha *et al.* (2005) found that an addition of 0.1% guar gum to the *idli* batter has a significant batter stabilizing effect, and *idli* made from them after 10 days of room temperature and 30 days of refrigerated storage was found to be the best acceptable quality. Agrawal *et al.* (2000) reported that the use of defined microbial starter cultures in the fermentation of *idli* batter preparation harbors desirable flavor compounds such as ketones, diols, and acids up to 8 days of storage, whereas undesirable flavors like sulfurous and oxazolidone compounds, ethanone, and thiazole appeared in the batter subsequent to 6 days of storage. The addition of essential oils (asafoetida, black pepper, white pepper, coriander, clove, cumin, fennel, garlic, ginger, parsley seed, and mustard) inhibits the overgrowth of microflora and increased the shelf life of *idli* batter. About 0.1% mustard oil added to the fermented *idli* batter harbor long shelf life (30 days) when stored at 4°C (Regubalan and Ananthanarayan, 2018). The growth of pathogenic bacteria in LAB fermented foods are often proven to be overwhelmed and with lower contamination levels (Adams and Nicolaides, 1997). *Idli* batter is commonly prepared by local traders and due to inappropriate handling, it is possibly spoiled with food pathogenic organisms.

1.6 Nutritional Value

In a nutritious food preparation, combination of cereal and legume is beneficial as it can be an enhanced diet balanced with both carbohydrates (cereal) and proteins (legume) (Blandino *et al.*, 2003). Additionally, legumes can also counterbalance the deficiency of the few amino acids in cereals. For instance, amino acids like lysine are absent in cereals while legumes are a rich source of lysine; consequently, the combined compensating those amino acid deficiencies (Nout, 2009).

Fermentation is an essential part of *idli* and *dosa* preparation as it enriches the nutrition and protein efficiency value (Reddy *et al.*, 1982). The process of *idli* and *dosa* preparation and fermentation completely alters the final quality with better flavor and texture, simultaneously with improved digestibility. Nutritionally *idli* and *dosa* are composed of carbohydrates, proteins, vitamins, and minerals (Rao, 1961; Rajalakshmi and Vanaja, 1967; Padhye and Salunkhe, 1978; Reddy *et al.*, 1982) (Table 12.2). As reported by previous studies (Kumar *et al.*, 2005; Balasubramanian and Viswanathan, 2007; Sridevi *et al.*, 2010; Manickavasagan *et al.*, 2013), employing varied proportions and variety of the rice and black gram changes the nutritional value and the quality of *idli*.

In *idli* and *dosa*, carbohydrates are highly available from rice and in addition black gram (Rao and Belavady, 1978) contributes to oligosaccharides (indigestible sugars such as raffinose, stachyose, and verbascose). The oligosaccharides are well known to produce intestinal gas and often removal of these sugars has been a challenging one (Rackis, 1981). During the process (soaking, fermentation, and steaming) of *idli* preparation, the oligosaccharide content was observed to decrease up to 34%. These indigestible sugars act as a fermentable substrate and include prebiotic properties that facilitate the growth of LAB (Chen *et al.*, 2007; Martínez-Villaluenga and Gómez, 2007).

Black gram is the main supplier of protein in *idli*. Further, in the fermented *idli* ratio of protein efficiency is higher than in unfermented *idli* batter (Veen *et al.*, 1967). Irrespective of the rice and black gram ratio, a notable increase in amino acids was also marked throughout the fermentation. Particularly, the concentration of methionine increased between 10% and 60% of the original content, although the reason for the increase is still unknown. As most of the legumes are deficient in sulfur-containing amino acids, an increase in methionine content was nutritionally beneficial (Reddy *et al.*, 1982).

Idli fermentation improved B-vitamins, particularly folate, pyridoxine, niacin, riboflavin, thiamine, and vitamin B_{12} (Reddy *et al.*, 1982). Balasubramanian and Viswanathan (2007) showed a two- to threefold higher B-vitamins content after fermentation. In addition to B-vitamins from rice and black gram, LAB and yeast further improve the content during the fermentation process. The indigenous strains were isolated from the fermented *idli* batter and tested for vitamin production efficiency. Yeasts are reported to involve in the synthesis of folate, riboflavin, and thiamine (Soni and Sandhu, 1991), while LAB for the synthesis of vitamin B_{12} (Iyer *et al.*, 2011). The high levels of amylase activity, production of B complex vitamins and free amino acids were achieved in the yeast-enriched fermentation process, which suggests the beneficial effect of yeasts on the production of these constituents (Venkatasubbaiah *et al.*, 1984, 1985). Recently, it was demonstrated that *Lactobacillus plantarum* isolated from *idli* produced 8 µg/mL of vitamin B_{12} and plantaricin LP84 (a bacteriocin) which harbor probiotic properties (Iyer *et al.*, 2011).

TABLE 12.2

Nutritional constituents of *idli* and *dosa*

Constituents	Unfermented batter	Fermented batter	Steamed *idli*/ Roasted *dosa*
Arginine	6.9 (mg%)[1]	8.9 (mg%)[1]	ND[1]
Ash	–	–	38.2 g (%)[2]
Calcium	0.4 (mg/g)[3]	0.4 (mg/g)[3]	
Carbohydrate	8.1 (g)[2]	7.4 (g)[2]	8.2 (g)[2], 77.6 (g/ 100)[4]
Choline	0.324 (g%)[11]	–	0.434(g%)[11]
Cysteine	0.2 (mg%)[1], 0.24 (g%)[5]	Trace[1], 0.022 (g%)[5]	ND[1]
Fat	–	–	0.1 (g)[2], 1.26 g[4]
Fiber	–	–	1.42 (g/100g)[4]
Folic acid	0.48 (mg/100 g)[6]	0.70 (mg/100 g)[6]	
Inorganic phosphorus	0.4 (mg/g)[7]	1.1 (mg/g)[7]	1.4 (mg/g)[7]
Inorganic Sulphur	1.3 (mg/g)[7]	0.3 (mg/g)[7]	0.3 (mg/g)[7]
Iron	46.9 (mg/g)[3]	46.4 (mg/g)[3]	3.8 (mg/g)[2]
Lysine	4.8 (mg%)[1]	6.2 (mg%)[1]	ND[1]
Magnesium	1.4 (mg/g)[3]	1.4 (mg/g)[3]	–
Metheonine	1.0 (mg%)[1], 0.129 (g%)[5]	1.6 (mg%)[1], 0.136 (g%)[5]	ND[1]
Moisture	–	–	50.5 (%)[2]
Phosphorous	3.7 (mg/g)[3]	3.7 (mg/g)[3]	–
Phytate	6.14 (g/kg)[9]	4.52 (g/kg)[9]	–
Phytic acid	2.5 (mg/g)[7], 174 (mg%)[8]	1.5 (mg/g)[7], 113 (mg%)[8]	1.5 (mg/g)[7]
Protein	13.9 (g)[8]	14 (g)[8]	3.3 (g)[2], 12.07 (g/ 100g)[4]
Raffinose	ND[7]	ND[7]	ND[7]
Riboflavin	0.42 (mg/100 g)[6], 0.25 (mg%)[8]	0.75 (mg/100 g)[6], 0.54 (mg%)[8]	–
Stachyose	3.1 (mg/g)[7]	2.9 (mg/g)[7]	2.4 (mg/g)[7]
Sucrose	8.3 (mg/g)[7]	5.6 (mg/g)[7]	5.1 (mg/g)[7]
Thiamine	0.46 (mg/100 g)[6], 0.21 (mg%)[8], 26.8 (mg/kg)[9]	0.72 (mg/100 g)[6], 0.58 (mg%)[8], 36.9 (mg/kg)[9]	–
Total phosphorus	3.7 (mg/g)[7]	3.7 (mg/g)[7]	3.7 (mg/g)[7]
Tryptophan	3.6 (mg%)[1]	3.3 (mg%)[1]	ND[1]
Verbacose	15.5 (mg/g)[7]	12.7 (mg/g)[7]	10.3 (mg/g)[7]
Vitamin B_1	0.24 (mg/100g)[10]	0.44 (mg/100g)[10]	0.19 (mg/100g)[10]
Vitamin B_2	0.26 (mg/100g)[10]	0.47 (mg/100g)[10]	0.30 (mg/100g)[10]
Vitamin B_{12}	3.9 (ng%)[11]	–	3.7 (ng%)[11]
Zinc	23.5 (mg/g)[3]	23.4 (mg/g)[3]	–

[1] Padhye and Salunkhe (1978),
[2] Sridevi *et al.* (2010),
[3] Reddy and Salunkhe (1980a),
[4] Uma Maheswari and Shetty (2013),
[5] Steinkraus *et al.* (1967),
[6] Ghosh and Chattopadhyay (2011),
[7] Reddy and Salunkhe (1980b),
[8] Rajalakshmi and Vanaja (1967),
[9] Shrivastava and Ananthanarayan (2015),
[10] Padmashree *et al.* (2014),
[11] Rao (1961).

The nutritional composition depends upon the ratio of raw materials (rice and black gram).

Also, *idli* contains minerals such as calcium, iron, magnesium, phosphorous, and zinc. During fermentation, levels of zinc and iron increased significantly from 50% to 276%. Organic acids and phytases produced by microorganisms are involved in hydrolysis that ends in the formation of ligands of iron and zinc (Hemalatha *et al.*, 2007). Apart from these, antinutritional factors such as phytic acid, saponin, flatus sugars, and trypsin inhibitors are also reduced in *idli*, all of which are mostly contributed by the black gram (Shrivastava and Ananthanarayan, 2015). About 35–40% reduction of antinutritional factor levels in *idli* was observed during the process of *idli* preparation (Reddy *et al.*, 1982). Similarly, fermentation and steaming of *idli* batter result in the complete reduction of enzyme inhibitors and the chymotrypsin inhibiting activity (Reddy *et al.*, 1982; Steinkraus *et al.*, 1996). Several volatile flavor constituents belonging to ketones, aldehydes, esters, organic acids, and heterocyclic compounds were also identified (Agrawal *et al.*, 2000) in *idli* batter. *Dosa* is reported to possess a significant amount of various nutrients (free amino acids, organic acids, proteinases, amylase, folic acid, vitamin B_1, and vitamin B_2), along with antimicrobial and antioxidant compounds. The fermentation process of *dosa* batter increases the mineral (zinc and iron) content and other essential nutrients and decreases antinutrient factors (Gupta and Tiwari, 2014).

1.7 Health Benefits

Probiotic assessment of LAB from *idli* batter has been extensively studied by a lot of researchers (Iyer *et al.*, 2011). Isolates from the *idli* batter with functional traits such as exopolysaccharides (EPS) and bacteriocins was illustrated by several authors (Agaliya and Jeevaratnam, 2013). LAB genera, including *Lactobacillus*, *Weissella*, *Pediococcus*, and *Leuconostoc* sp., from *idli* batter were reported for EPS production (Patel *et al.*, 2012; Das and Goyal, 2014; Kavitake *et al.*, 2016; Saravanan and Shetty, 2016).

Idli is generally consumed for losing weight and for anti-obesity. In addition, it also functions as a nutritional supplement for children suffering from malnutrition and Kwashiorkor. Reduction in the risk of high blood pressure, cardiovascular diseases, and stroke are added advantage. A substantial increase in the bioavailability of zinc (50–71%) and iron (127–277%) content under *idli* consumption is observed by Hemalatha *et al.* (2007). During *idli* batter fermentation, production of several bioactive compounds (thiamine, methionine, choline, and riboflavin) and an increase in nutrient and protein content is reported by Ghosh and Chattopadhyay (2011). Bacteriocinogenic *Pediococcus pentosaceus*, a xylose-utilizing *Pichia kudriavzevii* and *Weissella confusa* strains from *idli* batter possess excellent probiotic characteristics such as resistance towards bile salt, gastric and intestinal conditions, strong auto-aggregation and co-aggregation, and higher cholesterol assimilation (Vidhyasagar and Jeevaratnam, 2013; Chelliah *et al.*, 2016; Sharma *et al.*, 2018). Interestingly, a unique low-molecular-weight (2-hydroxyl indole-3-propanamide) antimicrobial substance produced by *Lactobacillus* and *Pediococcus* strains isolated from *idli* batter display antimicrobial activity against several pathogenic bacteria while sparing existing probiotic organisms (Sadishkumar and Jeevaratnam, 2017). In addition, probiotic microorganisms such as *Lactobacillus plantarum* and *L. lactis* isolated from *idli* batter produce vitamin B_{12} and β-galactosidase enzyme, which enhance the probiotic activity and promote health (Iyer *et al.*, 2011).

A backslopping method for accelerating the *idli* fermentation process was developed by Shrivastava and Ananthanarayan (2015), which enriched the thiamine levels along with a reduction of phytate and trypsin inhibition. Similarly, soy milk fermentation performed using LAB isolates from *idli* batter considerably reduced the polyphenols and trypsin inhibition, whereas increasing the phytase production and antioxidant property (Sadishkumar *et al.*, 2015). Potent LAB isolates from *idli* batter displayed antioxidative property, good adhesion and pathogen inhibition against human colon cancer cells (Sadishkumar and Jeevaratnam, 2017). A LAB (*Lactobacillus plantarum*) strain isolated from *dosa* exhibited significant antimicrobial activity against foodborne pathogens. *Dosa* has a low level of glycemic and glycemic index that lead to cure the problems associated with rheumatism, diabetics, and neural disorders. (Gupta and Tiwari, 2014).

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Section 4

Animal-Based Fermented Food Products

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13

Fermented Meat Products: From the Technology to the Quality Control

Maria João Fraqueza
CIISA, Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda, Lisbon, Portugal

Luis Patarata
CECAV, Animal and Veterinary Research Center, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Introduction

Fermented meat products have been historically associated with the need to preserve meat. Several preparations were developed worldwide, which became deeply rooted in the gastronomic culture of several regions of the globe. The manufacturing of fermented products was, and still is, associated to rural contexts of self-provisioning and local markets, contributing to the sustainability of the agro-food sector, particularly in mountain regions. To supply a greater number of urban consumers, fermented meat products are nowadays produced at industrial scale in different types of facilities. This manufacturing diversity and marketing dimensions inevitably has consequences on product characteristics that are available to the consumer, as well as their prices. 'Industrial' term is eventually used as a criticism, suggesting a product of lower quality however with a lower price. The price of dry-fermented meat products might differ tenfold according to the way of manufacturing, be it industrial or traditional. It is then not expected to have similar sensory quality parameters between these products (Fraqueza and Patarata, 2018).

The designative 'fermented meat product' encompasses any processed product that uses meat as the main raw material, and that supports the growth and activity of beneficial microorganisms adding value to the product, both by contributing for its sensory characteristics and/or for its preservation and safety. In Europe, according to EC Regulation 853 (2004), fermented products are included in meat products defined as '(…) processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat'.

The food safety and inspection services from the United States Department of Agriculture (USDA) defined fermented sausages that '(…) demonstrate a product of lower pH due to fermentation, followed by weight loss and decrease in water activity during drying. These parameters vary in their rate and extent depending upon the specific formulation and process' (FSIS/USDA, 2018). In the vast majority of the cases, fermented meat products are also dried in different extent. From the interaction of fermentative activity, moisture loss during drying, and eventual use of mild heat heating treatments, results the product characteristic, with adequate preservation parameters and sensory attributes (Cadavez *et al.,* 2016).

Fermented meat products is an umbrella designation, which includes products made with or without addition of starter cultures, fermentable carbohydrates, and temperatures in the beginning of the process that helps the fermentative microbiota multiplication. Those products that have a spontaneous fermentation of meat result from microbiota naturally added and are present on fresh meat and other ingredients. Also in some regions a well-known practice has been the addition of a piece of a previous batch to the ingredients of a new batch (backslopping) (Fraqueza, Patarata and Lauková, 2016). The starter culture might be defined as the preparation of viable cells of bacteria, yeasts, or molds that have particular metabolic activities, particularly the fermentation of carbohydrates and consequent acidification, and contributes to development of colors and aroma of the fermented meat products. Nowadays, the designation starter culture and protective or bioprotective cultures are all used to refer to these preparations used in the manufacture of fermented meat products. In the second group, the cultures that have a proved antimicrobial effect against undesirable bacteria, particularly pathogens, are included (Oliveira *et al.,* 2018).

Technology Factors with Impact on Fermented Meat Products Quality and Safety

A generic workflow of fermented meat products process is shown in Figure 13.1. Meat is prepared, cut or minced in appropriate pieces, seasoned and added with additives. In this stage, a starter culture and carbohydrates could also be added. If these last ingredients were not added, the meat still will suffer a spontaneous fermentation (Ojha *et al.,* 2015). The mixture might rest in cold storage before stuffing, or be immediately stuffed. After stuffing, the products are slightly heated to temperatures usually between 15°C and 22°C, eventually 26°C, that favors the growth of beneficial microorganisms—those included in starter cultures or those naturally present in the raw materials. After this initial stage of fermentation, products are kept during several days to weeks in conditions of controlled temperature and relative humidity to dry until they reach an adequate activity of water (aw) (García-Díez and Patarata, 2017). In some industrial methods, after the initial fermentation, and before or concomitantly with the drying, the products are pasteurized, to guarantee the elimination of pathogens and to develop specific sensory characteristics, which are different from the products only dried. The final products, dried to a specific level, will be packaged, labeled, and stored until expedition (CFIA, 2018).

Several factors could influence the quality of this final product from the formulation with different ingredients, the ingredients itself and their concentrations, the microbiota, and process parameters such as temperature and relative humidity.

Raw Materials: Meat and Fat

The quality of meat is a key factor for a successful manufacturing of dry fermented meat products. Besides safety and the hygienic conditions of the meat, which are assured by the official inspection and good slaughtering practices, rapid cooling, refrigeration during storage and transport, the intrinsic quality of meat, namely its suitability to drying process, must be considered. Low initial counts of microorganisms are preferred, which will be easier for the fermentative microflora to install and dominate the ecosystem, reducing the odds of having abnormal fermentations due to undesirable microorganisms (Leistner, 1992). The maximum microbial counts on meat should be defined by each producer, according to their own criteria for quality and safety (Regulation CE 852, 2004). It is recommended to avoid the use of meat with more than 2 log CFU/g of total viable microorganisms, and, even for those manufactures less exigent, the initial counts should never be higher than 4 log CFU/g (Conter *et al.,* 2007). The

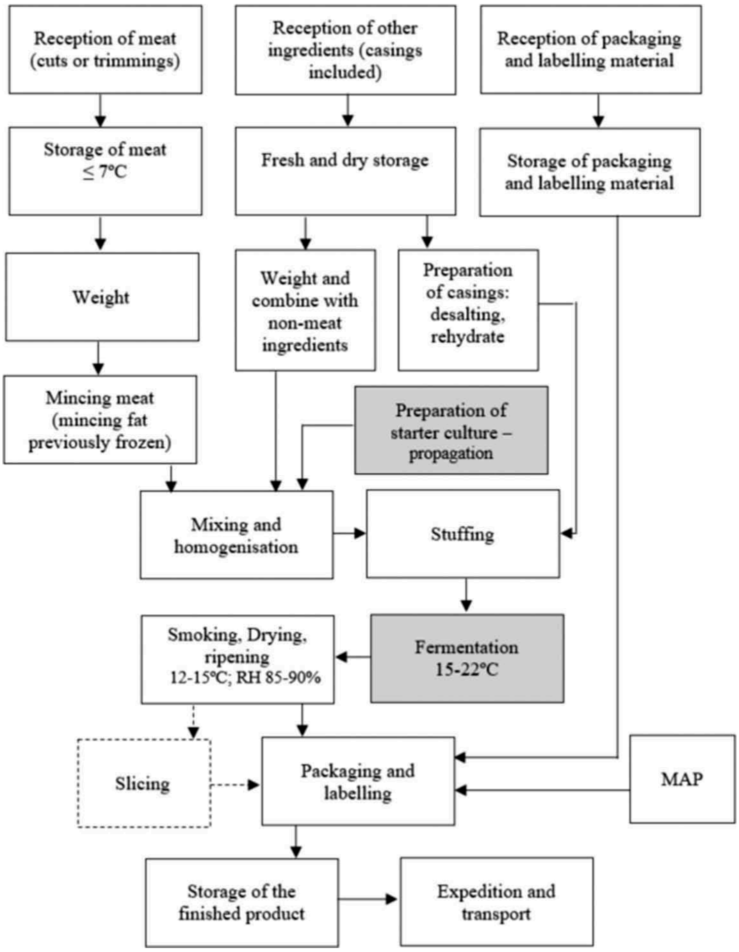
Reception of meat (cuts or trimmings)
Reception of other ingredients (casings included)
Reception of packaging and labelling material
Storage of meat ≤ 7°C
Fresh and dry storage
Storage of packaging and labelling material
Weight
Weight and combine with non-meat ingredients
Preparation of casings: desalting, rehydrate
Mincing meat (mincing fat previously frozen)
Preparation of starter culture – propagation
Mixing and homogenisation
Stuffing
Smoking, Drying, ripening 12-15°C; RH 85-90%
Fermentation 15-22°C
Slicing
Packaging and labelling
MAP
Storage of the finished product
Expedition and transport

FIGURE 13.1 General flowchart for fermented meat products process.

ability of meat for drying process is critical to obtain good quality products. Two major aspects can be considered—the weight of the carcass, amount and type of fat, and the occurrence of meat with abnormal quality characteristics, namely being pale soft and exudative (PSE) or dark, firm and dry (DFD) (Haddad *et al.,* 2018). Nowadays, the intensive pig production is aimed to obtain raw meat for direct consumption. Animals are slaughtered with light and lean carcass weight to fulfil the consumer demand for very lean and tender meat, and to maximize the feed yield. That meat is inadequate to manufacture raw dry sausages, once its drying kinetics if very fast and the low amount of fat compromises the juiciness and the flavor of the product. The meat color is expected to be paler. These concerns are also applicable to PSE meat. On the other hand, the main concern of DFD meat is its potential ability to support spoilage microorganisms and pathogens better than normal pH meat, due to its higher final pH (Toldra, 2007; Cierach and Modzelewska-Kapituła, 2012).

In the selection of meat for high quality products, it will be preferable to use indigenous breeds, and animals created under the so-called extensive or semi-extensive conditions which, by allowing the physical exercise of the animals, will result in meats of a more suitable color, fat interspersed and infiltrated between the muscular fibers (intramuscular fat, marbling), and a firmer texture

These animals often have a feed based on local products that provide unique characteristics of the meat, particularly the fat, as is the case with the acorn-finished pigs (Cava *et al.,* 1997; Teixeira and Rodrigues, 2013; Laranjo *et al.,* 2015).

The preparation of the meat and fat is an important step since the diameter size of pieces obtained from cutting or gridding could give us different specifications for final product quality and influence appearance, drying step, and final texture. The size of the pieces could be variable among products. It can vary from single piece, like 10 to 15 cm, in several products made from pork loin, like Portuguese *salpicão* and *paio* or Spanish *lomo embuchado* to small pieces of 0.5 cm in Italian-like salami (Choe *et al.*, 2014; Elías and Carrascosa, 2010).

Seasoning

There are several seasonings accepted in the manufacturing of fermented meat products, but the salt is probably the only one that is obligatory. Its effect in the taste of the product and the contribution to myofibrillar proteins solubilization is crucial for the texture and cohesion of the sausage. The solubilization of myofibrillar proteins promoted by the salt results in a sticky gel that together with the dehydration contributes to the link of the pieces of minced meat, and between them and the fat, resulting in a firm and regular texture, allowing the posterior slicing of the products (Inguglia *et al.,* 2017; Stanley *et al.,* 2017). The seasoning viewing the aromatization might include garlic, pepper, bay leaf, nutmeg, clove, among others. Wine is currently used as seasoning in regions with a rooted tradition in wine making (Linares *et al.,* 2013). In the last few years, a considerable amount of research was published on the interest of using essential oils from spices and herbs in spite of the original seasoning. These researches were conducted viewing mainly to take advantage of the antimicrobial effect of the essential oils; however, they have generally significant negative impacts on the sensory characteristics of the products, hindering its use in commercial conditions (García-Díez *et al.*, 2016; García-Díez and Patarata, 2017), in addition to the eventual problem of toxicity they might present (Oliveira *et al.,* 2018).

Additives

There are several food additives that could be used in the manufacturing of fermented meat products. The list of chemicals additives that can be used in meat products is usually quite short, and includes the molecules previewed by law in different economic areas. As globalization has hit the market, there are guidelines made by the Joint Expert Committee on Food Additives of FAO/WHO (JECFA, 2018). In the present revision we will focus on those additives that have a key role on the manufacturing of fermented meat products. Nitrite is probably the most important additive used in the manufacture of meat products, and simultaneously the additive that has a more questionable use, due to its potential negative effects on consumer's health (Holck *et al.,* 2017; Mortensen *et al.,* 2017). Nitrite is used with a double function. To confer the characteristic of cured-red color to the meat products, and to control the eventual growth of pathogens, particularly the growth and toxin production of *Clostridium botulinum*. Sodium or potassium nitrite is reduced to nitric oxide, which is the reactive compound involved in both colours formation and microbial inhibition. Nitric oxide bonds to iron in the myoglobin molecule of meat, forming the pigment nitrosomioglobin (Sebranek and Bacus, 2007; Hospital *et al.*, 2014; Hospital *et al.,* 2015). It is also the nitric oxide that contributes for the inhibition of *Cl. botulinum*, by the bonding to the iron–sulfur enzyme complex that interferes with the bacteria metabolism and compromises its survival (Majou and Christieans, 2018)

Casings

Most fermented meat products are sausages that might be stuffed in different types of edible casings. Natural pork casings, from small or large intestine, are traditionally used to prepare fermented sausages, but casings from other livestock animals can be used. Also, artificial casings made from collagens are currently used. The natural casings are currently preferred to the artificial ones due to its excellent ability to dry, elasticity, and natural aroma (Arslan and Soyer, 2018; Walz *et al.,* 2018). The natural casings are available in the market, calibrated and disinfected, packed in dry salt or in brine, with a good microbial profile. Artificial collagen casings might be more adequate for industries, producing large amounts of sausages, due to its regular caliber and easier adaptation to stuffing machines. Natural casings are more adequate for sausages in feet-horse-shape and artificial collagen casings for rectilinear ones (Wu and Chi, 2007)

Preparation of the Batter and Fermentation

In this stage of processing, there could occur the main differences between the various types of fermentation. Figure 13.2 illustrates the different methods of fermentation with commercial importance in meat products.

Meat is mixed with seasonings and additives (Laranjo *et al.,* 2015; García-Díez *et al.*, 2016). If the product suffers only a **spontaneous fermentation**, nothing else is added. The batter might rest at low temperature from 24 to 48 hours, before stuffing. This is particularly important when products are made from big pieces of meat to allow the distribution of the seasonings and additives, or it is immediately stuffed, particularly if it is produced from finely minced meat. During the initial period after mixing, the highly adapted lactic acid bacteria (LAB) naturally present in the meat and belonging to the house-microflora of the workshop or industry will grow and it is expected that they became the dominant microbiota after the first two or three days (Laranjo *et al.,* 2017b; Wang *et al.,* 2018). The main problem associated to spontaneous fermentations is the unpredictability composition of fermentative microbiota, due to the variability in the profile of the LAB associated to meat, and to the reduction in house microflora equipment and environment, due to the disinfection practices that could be nowadays very effective. Fermented meat products with starter cultures, hereafter designated as **induced fermentation**, are made as indicated above, but a starter culture is added to the batter, to guarantee that a known microorganism, or ensemble or them, dominate the microbial ecosystem of the batter and further the meat product, avoiding the occurrence of having unsuitable strains growing in the product (Woraprayote *et al.*, 2016; García-Díez and Patarata, 2017; Cruxen *et al.,* 2018).

In induced fermented products, it is common to add fermentable carbohydrates to the batter, in order to allow a rapid growth of the specific LABs and to promote the pH reduction, in the extension previewed for each specific product. To promote the implementation of the desired microbiota and to achieve a rapid pH decrease, glucose is usually used in fermented products, once this hexose is rapidly fermented by any LAB, mixed with a disaccharide, like lactose or sucrose, that has a slower fermentation. The temperature used in the fermentation step might also quite vary (Fraqueza *et al.,* 2016). Spontaneous fermentation might occur at low temperature, near 15°C or below, when products are only dried without smoke. When meat products are smoked in traditional smokehouses, the heating to warm temperatures is inevitable, and helps the growth of microflora with technological interest. Meat products inoculated with starter cultures might be submitted to a fast fermentation, using higher amounts of carbohydrates and higher temperatures, to achieve a pH enough reduced in a short period of time. To prevent the eventual overgrowth of undesirable microorganisms in the initial phase of fermentation, some industrial procedures use glucono-delta-lactone, which hydrolyses immediately when in contact with the water, forming gluconic

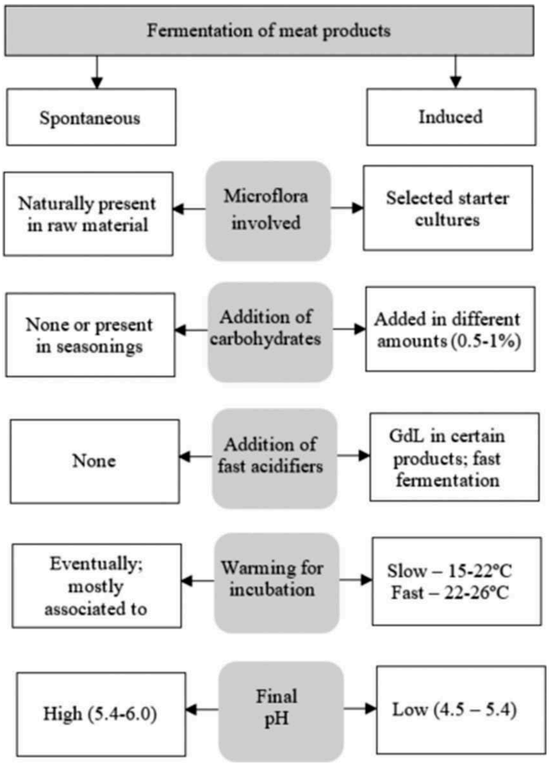
Fermentation of meat products
Spontaneous
Induced
Naturally present in raw material
Microflora involved
Selected starter cultures
None or present in seasonings
Addition of carbohydrates
Added in different amounts (0.5-1%)
None
Addition of fast acidifiers
GdL in certain products; fast fermentation
Eventually; mostly associated to
Warming for incubation
Slow – 15-22°C
Fast – 22-26°C
High (5.4-6.0)
Final pH
Low (4.5 – 5.4)

FIGURE 13.2 Main differences between spontaneous and induced fermentation of meat products.

acid, which immediately reduces the pH, while the starter culture is still in the lag phase (Yilmaz and Zorba, 2010).

Starter cultures can be added to the batter in different forms. The most commonly used is lyophilized to be added directly to the meat mixture. It is safer, once the probability of starter culture contamination it is nearly null. An older method consisted in propagating the cultures, usually in skim milk and then added the culture already grown to the meat. With this technique, it is necessary to have laboratory conditions in the workshop, to guarantee that the culture under propagation will be not contaminated. An ancient method to inoculate meat products with 'starter cultures' is the back-slopping. A portion of fermented product is added to the meat mixture to begin the fermentation. This technique is interesting to keep the house microflora of small workshops, but carries some risks, namely of inoculating with pathogens present in the former fermented batch (Fraqueza *et al.,* 2016).

The range of pH decrease influence several aspects of the fermented meat products. On one hand, the pH reduction is very attractive from the point of view of microbial hazards control. The lower the pH the slowest will be growth of the majority of the pathogens, and for most of them the low pH might have a lethal effect. On the other hand, the acidification imparts an acid taste to the product and might inhibit several biochemical phenomena involved in flavor generation, resulting in products with sensorial fails (Oliveira *et al.,* 2018).

Smoking

Traditional smoking, carried out in small manufacturing units, where wood or wood chips are used, can be carried out in two ways—cold and hot smoking. In both cases smoke is generated by burning wood, or wood chips. In the cold smoke environment temperatures usually do not exceed 20-30ºC, while in hot smoking, temperatures around 80ºC are chosen. In the vast majority of fermented product, cold smoke is used, once the temperatures reached in hot smoke have a lethal effect on the microbiota of the product, fermentative included (Roseiro *et al.,* 2012). In smoked products, it is during the smoking that the rise in the temperature favors the multiplication of the fermentative microflora, particularly LAB, that has ideal oxi-reduction conditions to growth inside the product. On the other hand, those bacteria that growth better in the presence of oxygen, namely *Staphylococcus* and *Kokuria*, might be negatively affected due to the antimicrobial effect of the smoke (Lingbeck *et al.,* 2014).

In small industries the meat products are hung in the upper part of the chambers. At the bottom, usually on a lower floor, is the fire that releases smoke and reaches the food, passing through a grid that corresponds to the floor of the chamber. The control of the amount of smoke and the temperature relies mostly in the experience of the person in charge of the fire. In industrial facilities, still that model of smoking chamber is also used, it is more common to have electric generators of smoke, which area a component of the smoking system composed by an electric resistance that burns slowly wood chips without live fire. The smoke is aspirated by a set of pipes, it is cooled by passing through a cold water curtain, avoid to heat excessively the products, and allowing also to withdraw some of the potentially toxic polycyclic aromatic hydrocarbons (PAH). Once this method depends on pumps and fans to aspirate and distribute the smoke in the chambers, it is expected to have a more reliable smoking, once it has a better repeatability (Woods, 2003).

Alternatively, and due to the concerns related to the potential hazard that the PAHs represent to human health, the use of smoke condensates, or liquid smoke as they are commonly known, can be an interesting strategy. In the preparation of smoke condensates the wood is subjected to intense heat and is partially burnt and the smoke immediately placed in a condenser. Smoke is forced into refined vats and subsequently filtered to remove toxic components and carcinogenic impurities (Simko, 2005). Refined, purified and concentrated, the smoke condensate might have advantages when compared to traditional smoke. The composition of traditional smoke will always depend on several factors including moisture content, pyrolysis temperature, process duration and wood quality. The smoke condensate can be applied to meat products in a variety of ways, namely immersion, shower or atomization. In the last one, the smoke condensate is mixed with compressed air, which is vaporized in the product, similarly to recently obtained smoke (Lingbeck *et al.,* 2014).

In traditional facilities, during the smoking step occurs part of the dehydration, while in more equipped facilities, smoking is made in a chamber only to aromatize and color the surface, and the drying is further done in specific chambers (Feiner, 2006).

Dehydration

Dehydration is a simple and primitive process, which is practiced to preserve food from immemorial times. Numerous references are made to dried meat products, particularly in European Mediterranean region, Asia, Africa and South America (Talon *et al.,* 2007; Sánchez Mainar *et al.,* 2016). The dehydration of the meat in the open air ensures preservation by reducing aw, however, the texture properties might be adversely affected and it is necessary to well control the kinetic and extension of the operation to avoid the need of rehydration (Lucke, 1985). Dry fermented meat products, as well as other similar products produced worldwide, undergo partial dehydration, which, together with other processes, ensures the reduction of aw to values that guarantee stability, without sensorial attributes being compromised. This reduction of aw is a consequence not only of the water elimination but also of the higher concentration of solutes in the residual water. These products that are preserved mainly by the effect of reduced aw, but are ready to eat without rehydration are usually known as Intermediate moisture foods (IMF) (Leistner, 1992).

The dehydration process consists of the transfer of water from the food and its removal from the surface of the product in the form of vapor. The surrounding air will carry this water vapor and will recirculate in order that some water vapor could be removed reducing the content of relative humidity. Although this is a simple concept, food dehydration is a complex physical phenomenon. During this process energy is supplied to the food in the form of heat, increasing the water vapor pressure and its consequent migration. This internal transfer of moisture comprises a set of diffusion, convection, capillary flow processes and physical deformation, among others. The increase in water vapor pressure is an endothermic mechanism; but when it is withdrawn in the form of steam from the surface of the product, there is loss of heat, a phenomenon known as evaporative cooling. Accordingly, to maintain the product surface at a temperature high enough to ensure evaporation, continuous heating is required. During the dehydration process, the evolution of aw has no linear relationship to the residual moisture content. These two parameters are related through an isothermal water sorption (Feiner, 2006).

In the manufacture of dry fermented meat products, the success of dehydration process depends on several factors that can be grouped into extrinsic and intrinsic ones. In the first group are considered the environmental factors: relative humidity, air circulation velocity and temperature. The group of intrinsic factors encompasses the specific characteristics of each product, such as the casings permeability, the size of meat pieces, the fat content, the pH of the mixture, the presence of certain ingredients, among others. The salt content also influences the dehydration kinetics (Kumar *et al.,* 2017).

To control the process, it is difficult to manipulate the intrinsic factors, as they depend on the formulation of the product that gives its specificity. The extrinsic factors must be adjusted to the characteristics of each product to avoid a very fast drying, with negative repercussions on the texture properties, or a very slow drying, usually associated to the surface development of aerobic microorganisms, namely mold and yeasts, that might be undesired if they are not a regular specification of the product (Fraqueza and Patarata, 2018).

Although there is no consensus to the most effective relative humidity conditions, air velocity and temperature for meat products dehydration process, it has been found that the relative humidity should remain below the aw of the product, close to 70 to 80 %. Thus, the water can be eliminated from the surface of the product at a rate similar to that of the internal transfer of moisture. If the dehydration is very pronounced, it can cause a phenomenon known as case hardening, characterized by an excessive dehydration of the peripheral layer, with the consequent formation of a crust, which hinders the exit of water from the interior of the product. The air velocity also depends on many factors. If continuous dehydration it is advisable to maintain an air velocity below 0.05 ms^{-1}. In active/stop cycles, the air velocity may be increased. The dehydration temperature should be below 16°C, in order to reduce the fats oxidation and control the multiplication of some undesired microorganisms. The use of higher temperatures can cause a rapid dehydration, or else the cooking of the product, deeply modifying the organoleptic characteristics (Leistner, 1992; Feiner, 2006).

In small workshops, that on majority supply local markets, the manufacturing conditions are based on empirical know-how, without mechanization of several processing steps. In these workshops, drying takes place simultaneously with smoking being carried out in structures that have adapted the conditions of artisanal manufacture to a largest size of production. With this type of premises, it is very difficult to control the temperature at which the products are subjected, having a very important human component in the control of this operation, which is ensured by the person in charge of the fire for smoking production. The curing/stabilization is carried out in air-conditioned chambers, or in fresh parts of the factory (Morales-Partera *et al.,* 2017).

In large industries with a high level of mechanization, it is possible to strictly control all technological parameters, given the availability of smoking chambers and drying chambers with controlled relative humidity, air velocity and temperature (Simko, 2009).

Control of drying can be carried out by evaluating, during the course of the process, weight loss or aw. For companies with little financial capacity, weighing the products can be a good solution to control dehydration. Therefore, the manufacturer should for each produced batch, weigh a significant number of samples and calculate the degree of dehydration, relative to the initial weight. In order to use this process, the manufacturer must know the products well, since the degree of dehydration depends very much on the composition, particularly the abundance of fat. It is found that a product that loses half of its water by evaporation will lose about 38% and 4% of meat water and fat, respectively. In practical terms, the weight loss is usually between 25 and 40%. Direct control of aw is the best and faster procedure, and can be used in all batches as monitoring strategy or to gauge the necessary weight loss (Girard and Girard, 1988).

Concomitantly with the drying process, there are a number of biochemical and chemical modifications that, together with the reduction of moisture and pH modification are usually referred as ripening. In that stage, besides the use of carbohydrates in fermentation, major modifications occur in the protein and lipid fractions, that determines the final texture of the fermented sausage, but mostly its flavor (Demirok Soncu *et al.,* 2018).

In fermented meat sausages there are numerous modifications of the nitrogenous fraction during the fermentation and ripening process, both at the proteolytic and amino acid catabolism level, whose repercussions on sensorial characteristics are recognized, namely on products flavor. The modifications on the protein fraction are determined by both endogenous muscle enzymes and by those of microbial origin. Several studies have shown that the endopeptidase activity, responsible for peptides liberation is mainly from endogenous origin. As a consequence of the various modifications occurring in the nitrogen fraction, there is an increase in the peptide and free amino acid content (Paredi *et al.,* 2012; Zhao *et al.,* 2016). This increase in peptides and free amino acids might have a direct impact on the taste of the products, since the amino acids have specific flavors, namely bitter, attributed to hydrophobic L-amino acids, sweet, characteristic of alanine, glycine, serine and threonine, acid of the aspartic and glutamic acids or umami, characteristic of the sodium salts of the last two (Ordonez *et al.,* 1999). The increase in the free amino acid content in the food, besides the direct reflexes that have on the taste, can be in the base of the formation of other compounds with impact on the flavor, through the catabolic (or anabolic) activity of the microflora or by non-enzymatic processes. In some cases, the link between these processes is difficult to establish (Pérez-Santaescolástica *et al.,* 2018).

Lipids are eventually the most important fraction of fermented meat products. The modifications they undergo may be of hydrolytic or oxidative nature, which, although having a different origin, are closely related. Free fatty acids, resulting from the hydrolysis, are more prone to oxidation than when they are esterified in triglycerides. The hydrolytic modifications occurring during the ripening are due to the action of enzymes on ester bonds involving fatty acids (Alfaia *et al.,* 2015; Mariutti and Bragagnolo, 2017).

The contribution of the free fatty acids to the organoleptic characteristics of the sausage is unclear, as, unlike in dairy products, where the fat includes significant amounts of short-chain fatty acids, and which are responsible for acid-like sensory characteristics, the contribution of the free fatty acids to the flavor of meat products will be marginal, since the fatty acids released in greater quantity, particularly oleic and linoleic are considered devoid of smell or taste (Forss, 1969; Dainty and Blom, 1995).

Oxidation of fatty acids plays a key role in sausages and other cured meat products. On the one hand, these modifications are associated with derogatory sensorial characteristics, namely rancid smell, color changes, as well as the formation of potentially toxic compounds. On the other hand, in the work of identifying the components of the volatile fraction of the fermented sausages, the importance of the lipid oxidation compounds as the main responsible for their typical aroma is highlighted, not only for its abundance, but also for the reduced olfactory perception threshold has (Flores *et al.*, 2017; Flores, 2018).

Packaging and Storage

The packaging of fermented meat products is used to protect the product from contamination with several types of hazards from the exterior, that could reduce its safety, and also to avoid excessive drying, loss of aroma and prevent the surface growth of microorganisms. The packaging of fermented meat products might be done in vacuum package or modified atmosphere (Pereira *et al.,* 2015). The type of film used should be adequate to the finality. Some fermented meat products are packaged in open packages—net bags, paper based or plastic with holes, to avoid the eventual surface condensation of moisture and accumulation of gas produced by the fermentative microbiota.

The package is also the support to apply the label. In most countries the labelling is compulsory, in EU due to the Regulation 1169 (2011), where several information should be included, related to the producer, the raw materials used, eventual presence of allergens and nutritional information.

The storage of fermented meat products should be limited to the established shelf life that should have in consideration the loss on sensory characteristics and the eventual degradation of the food safety status (Hough and Garitta, 2012). Considering the reduced aw these products present, it is highly improbable that pathogens might growth. The main concern should be the eventual multiplication of superficial molds (Vet *et al.*, 2004; Iacumin *et al.,* 2009), or the occurrence of chemical modifications, namely the formation of biogenic amines or complex compounds involving the species obtained by the reduction of the nitrite and nitrate (Kumar *et al.,* 2015; Rohrmann and Linseisen, 2016).

Microbiota Role on Fermented Meat Products

Fermented meat products result from the presence of heterogeneous microbial groups of bacteria, yeasts and molds influencing sensory characteristics and product stability. The microbiota of fermented meat products is well adapted to the meat substrate but there is a considerable diversity according to several factors. The initial microbial consortium depends on intrinsic (nutrients, pH, redox potential, buffering capacity, aw, meat structure) and extrinsic factors (temperature, relative humidity and oxygen availability) of raw meat and other ingredients added on product formulation (sodium chloride, nitrate/nitrite, sugars, wine, condiments (garlic, pepper)), and afterwards it will be modulated along all technological process by temperature, time and relative humidity. Other factors will also modulate this fermentative microbiota: the cross contamination with environmental working surfaces and personal during meat preparation and ingredient mixing, the oxygen gradient before and after stuffing, ripening and smoking (Fraqueza *et al.*, 2015).

The main group associated with fermented meat products are the lactic acid bacteria (LAB). This group have been identified and studied in several fermented meat products from different origins (Figure 13.1), and there are main genera frequently found: *Lactobacillus, Enterococcus, Lactococcus, Pediococcus, Leuconostoc, Weissella.* This heterogeneous LAB group are Gram positive non-spore forming cocci, coccobacilli or rods, with common morphological, metabolic and physiological characteristics and belongs to the phylum Firmicutes, class *Bacilli*, and order *Lactobacillales* including mainly the genera *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Ludwig *et al.,* 2009; Vandamme *et al.,* 2014).

Lactic acid bacteria are classified as homofermentative or heterofermentative, according to their glucose metabolism end products. Their growth depends mainly on the presence of fermentable carbohydrates.

Acid production is a central process in the manufacture of fermented foods and is one of the primary roles of the fermentative spontaneous microbiota or if there is an application of particular starter cultures. The carbohydrate most commonly used in the fermentation is a hexose—glucose. It is also common to use disaccharides that by hydrolysis originate hexoses (sucrose, lactose, maltose) (Ferrer Valenzuela *et al.,* 2015). The choice for these carbohydrates depends on the desired kinetic and extension of the acidification. When a rapid reduction in pH is desired it is used glucose, which is readily metabolized. If a slower process and a less extensive acidification is desired, lactose or sucrose are usually the ingredients chosen (García-Díez and Patarata, 2017).

Lactic acid bacteria ferment hexoses mainly to lactic acid, under normal conditions of excess substrate and limited access to oxygen, and can follow three metabolic pathways, which are associated with specific microorganisms and whose end products are characteristic: (i) the Embden-Meyerhoff-Parnas (EMP), also referred to as the homofermentative or glycolytic pathway; (ii), the 6-phosphogluconate/phosphocetolase or heterofermentative pathway, and (iii) the fructose-6-phosphocetolase pathway, or bifidobacteria pathway (Ferrer Valenzuela *et al.,* 2015; Hansen, 2018). In the three mentioned routes, the two lactic acid isomers, (D) or (L), might be produced, depending on the stereospecificity of the lactic acid dehydrogenases (LDH) present, or the possible action of a racemase, which converts L-lactic acid formed at an early stage in D-lactic acid (Juturu and Wu, 2016). The EMP pathway is used by species of *Enterococcus*, *Lactococcus*, *Streptococcus* and some species of *Lactobacillus*, namely those integrated in the designated group I, obligatory homofermentative, and group II, optional heterofermentative (Kandler and Weiss, 1986). The 6-phosphogluconate/phosphocetolase pathway is used by obligate heterofermentative species of *Leuconostoc* and *Lactobacillus* of group III (Gottschalk, 1986). Finally, the Bifidobacteria pathway is used by bacteria of this genus.

Although, by definition, homofermentative LAB almost exclusively convert hexoses into lactic acid, there are several factors that can modify this pattern, namely the limitation or type of carbohydrates that supports the growth, the pH of the medium and the availability of oxygen. This modification from a fermentation commonly referred to as mixed or mixed acid is characterized by the concomitant production of lactic acid with other compounds, such as acetic acid, formic acid, ethanol, carbon dioxide. Its occurrence and individual abundance is fundamentally conditioned by metabolic determinants expressing not only the enzymes required in the microorganism but also biased by numerous regulatory factors (Hansen, 2018). The type of substrate that supports growth may also determine the deviation of the homolactic to the mixed fermentation. When lactose is used—which is the disaccharide supporting the fermentation of dairy products and also frequently added to fermented sausages—the glucose and galactose resulting from its cleavage can be catabolized by different routes. Thus, glucose is usually catabolized via the EMP pathway, and galactose via the tagatose pathway or via the Leloir pathway, depending on the lactose transport mechanism that was used (Thompson and Gentry-Weeks, 1994).

The implications of the formation of lactic and acetic acid on the characteristics of the fermented products are diverse, in addition to those related to the inhibition of undesirable microorganisms. In meat products, the reduction of pH reduces the water binding capacity of the proteins, favoring the dehydration. The abundance of lactic and acetic acids determines the peculiar flavor and aroma, due to their specific sour taste and *sui-generis* aroma (Ganzle, 2015). If produced in excess, they eventually determine abnormal flavor. For the D-isomer of lactic acid is assigned greater responsibilities in the unpleasant acid and sharp flavor of fermented foods. Acetic acid is considered to be an important component of the aroma typical of fermented sausages, when present in small amounts (Berdagué *et al.,* 1993). In excess, the vinegar aroma will dominate the flavor of the fermented sausage. Formic acid might also be formed in small amounts. Its impact on flavor is not well established (Dainty and Blom, 1995; Valenzuela *et al.,* 2015).

Some alcohols may also be formed from carbohydrates, namely ethanol and 2,3-butanediol, that have an impact on the aroma of the fermented sausage depending on the formed molecule (Raimondi *et al.,* 2018). Among the non-acid compounds formed on the metabolism of carbohydrates, 2,3-butanedione have an important impact in the aroma of fermented products, due to low olfactory perception threshold, giving a butter note on the flavor of the product that can be depreciative if its production is excessive. Though the production of 2,3-butanediol is known in some BAL used in the dairy industry (*Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp *cremoris*), it is also produced by *L. plantarum* that is currently found in fermented sausages (Ferain *et al.,* 1996).

The LAB species presented in Figure 13.3 play a recognized role in diverse fermented meat products preservation and safety, promoting their microbial stability. Common species are presented such as *L. sakei*, *L. plantarum*, *L. curvatus*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *E. faecalis*. Other species casually identified on fermented meat products could be associated with them. There are well adapted LAB strains on processing units' environment, constituting the so-called 'house microbiota', that are naturally introduced on meat fermented products during meat preparation and on different processing steps, being responsible for the spontaneous fermentation with production of lactic acid and other metabolites and for particularities regarding sensorial characteristics.

The production of organic acids by LAB give them also competitive advantage against other bacteria in the complex microbiota of fermented sausages. These organic acids inhibit the microbial growth through membrane disruption, inhibition of metabolic reactions, disturbance of pH homeostasis, and accumulation of toxic anions in the cell. Some LAB species will dominate because they have a greater acid tolerance and capability to adapt to redox changes than other bacterial species. Another important competition tool expressed in several LAB, namely in particular strains of *Lactobacillus, Enterococcus, Lactococcus, Pediococcus and Leuconostoc* are bacteriocins, that contribute to its dominance (Zacharof and Lovitt, 2012). Bacteriocins are a heterogeneous group of compounds of peptidic nature that exert an antimicrobial effect mainly by producing damages in the cell membrane. They are frequently associated to LAB and are considered to be safe since they can be degraded by proteases in gastrointestinal tract or even can have medical applications (Cleveland *et al.,* 2001). When applied on fermented sausages will give them a protection against relevant potential pathogens particularly *Clostridium botulinum, L. monocytogenes, S. aureus* and *Bacillus s*pp. (Woraprayote *et al.*, 2016, Fraqueza *et al.,* 2016).

Species *L. sakei*, *L. plantarum* and *L. curvatus* are particularly well adapted to meat substrate (Zagorec and Champomier-Verges, 2017) due to its specific genetic repertoire, that seems to be not expressed on other lactobacilli. These species present different energy production pathways, versatile redox metabolism conferring them a great resilience against different redox and oxygen conditions that are found in fermented meat products. The ability for biofilm formation and cellular aggregation contributing to their persistence on industry environment is also an important attribute leading to its dominance of the meat ecosystem (Guilbaud *et al.,* 2011; Muscariello *et al.,* 2013; Aoudia *et al.,* 2016).

Other LAB specie that could be present on fermented meat product are enterococci. The presence of *Enterococcus faecium*, *Enterococcus faecalis* or *Enterococcus durans* is cause of discussion since its presence can be seen as a lack of good hygiene practices and indicators of fecal contamination of meat products. Also, these enterococci are highly related with the production of biogenic amines such as tyramine and phenilethylamine (Alfaia *et al.,* 2018). However they can be associated with flavor development and are frequently mentioned as producers of bacteriocins (Callewaert *et al.,* 2000; Mareková *et al.,* 2007).

All the diversity at strain level inside the dominant species is important since could be responsible by specific characteristics related to products quality or safety. Figure 13.4 shows the evolution of LAB counts in a Portuguese smoked fermented sausage. After stuffing the LAB count increased till approximately 10^7 cfu/g, and during all the ripening period this level of LAB counts was maintained. This increase of LAB population is related to the decrease of pH till 5.00 observed in the first five days of fermentation (Figure 13.5), with a slightly increase during ripening period to a pH of approximately 5.2 in the final product.

The coagulase–negative cocci (CNC) or gram-positive-catalase-positive cocci (GCC+/CPC), is another main group of the microbiota present on fermented sausages, with elements from *Staphylococcus* and *Kocuria* genera. Once Staphylococcus is the main genera associated to the technological effect on meat products, hereafter we will refer to them as coagulase negative

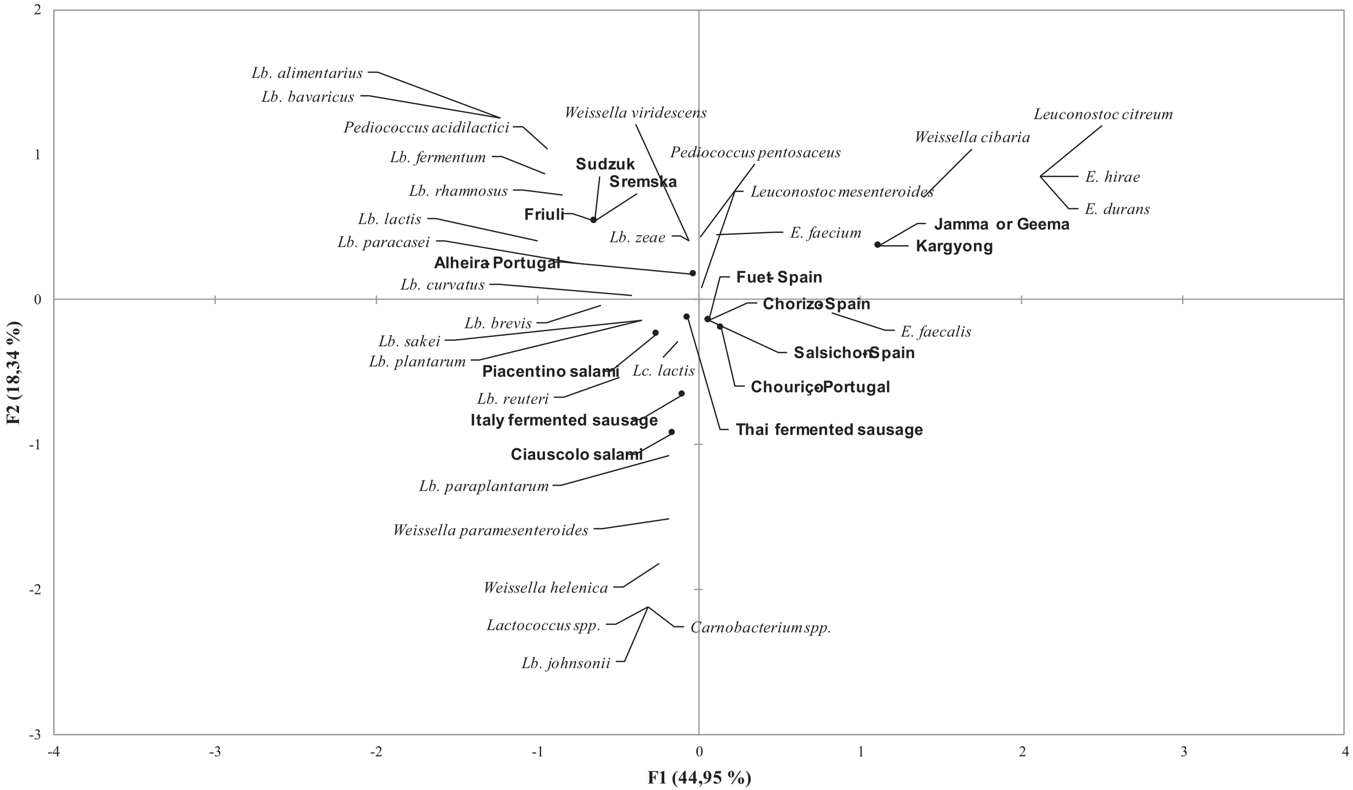

FIGURE 13.3 Major LAB species associated to diverse fermented meat products from different origins.

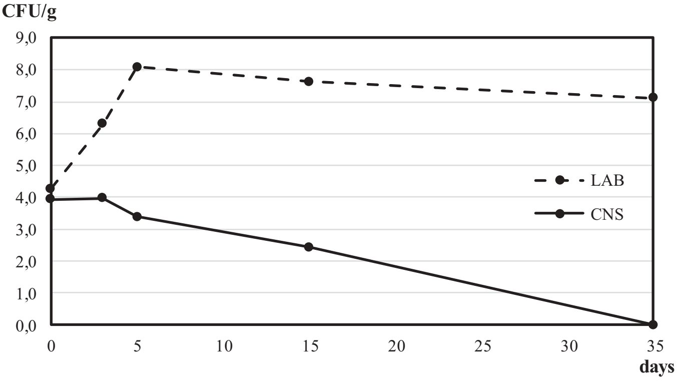

FIGURE 13.4 Evolution of LAB and CNS counts in a Portuguese smoked fermented sausage. Unpublished data. 0 days—after mixing; 3 days—after resting at 4ºC; 5 days—after smoking (maximum temperature 30ºC; 15 days—10 days drying (15ºC, 85% RH); 35 days—30 days drying (15ºC, 85% RH).

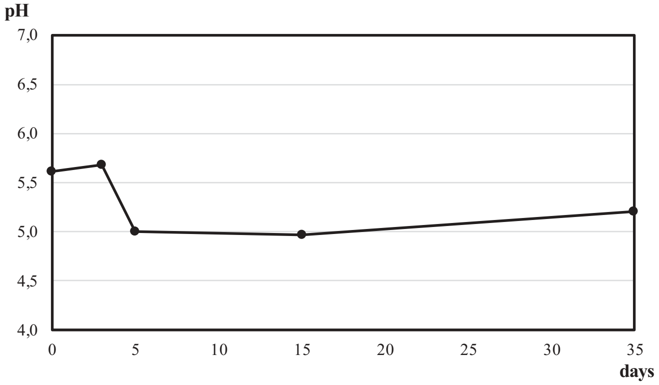

FIGURE 13.5 Evolution of pH in a Portuguese smoked fermented sausage. Unpublished data.0 days—after mixing; 3 days—after resting at 4ºC; 5 days—after smoking (maximum temperature 30ºC; 15 days—10 days drying (15ºC, 85% RH); 35 days—30 days drying (15ºC, 85% RH).

staphylococci (CNS). These are responsible by fermented sausages color and flavor development, by the presence of a nitrate-reductase activity, catabolism of free amino acids and inhibiting the oxidation of unsaturated free fatty acids (Leroy *et al.,* 2006). Therefore, this group is involved on proteolytic and lipolytic processes and is responsible by the organoleptic characteristics of fermented meat products. From *Staphylococcaceae* family the most frequently isolated species are *Staphylococcus xylosus*, *St. equorum*, and *St. saprophyticus* (Drosinos *et al.,* 2005; Fontana *et al.,* 2005; Corbière Morot-Bizot *et al.,* 2006; Fraqueza *et al.*, 2018). However, other species could be present being identified in several traditional products such as *Staphylococcus carnosus, Staphylococcus succinus*, *Staphylococcus epidermidis*, *Staphylococcus simulans, Staphylococcus haemolyticus*, *Staphylococcus pasteuri*, and *Staphylococcus warneri* (Iacumin *et al.*, 2012; Patarata, 2002; Corbière Morot-Bizot *et al.,* 2006; Coton *et al.,* 2010; Leroy *et al.,* 2010; Marty *et al.,* 2012; Ratsimba *et al.,* 2017*).* The modulation of this CNS group will be done along the processing of a fermented meat product (Leroy *et al.,* 2014). These microorganisms are the most efficient nitrate reducing microorganisms (Gøtterup *et al.,* 2008), and their presence are fundamental to permit the development and stability of the color of fermented meat products. *S. xylosus* and *S. carnosus* were described as improving the development and stability of the characteristic red color of fermented meat products because are able to reduce nitrate to nitrite through their nitrate reductase activity (Talon *et al.,* 1999). Subsequently, nitrite can be converted to nitric oxide (NO), which binds to the myoglobin ferrous heme-iron forming the stable red nitrosomyoglobin pigment (Gøtterup *et al.,* 2007, 2008).

The evolution of CNC counts in a Portuguese smoked fermented sausage can be observed on Figure 13.4. After stuffing the count of CNS in the sausage is high (10^4 cfu/g) but along drying, smoking and ripening period this group will decrease.

The catabolism of the branched amino acids, leucine, isoleucine and valine is at the origin of compounds which have been attributed important responsibilities in the typical aroma of fermented sausages, respectively 3-methylbutanal, 2-methylbutanal and 2-methylpropanal, such as alcohols and acids. The catabolic process of leucine, have received particular attention given the high importance that 3-methylbutanal, 3-methylbutanol and 3-methylbutanoic acid reveal for the characteristic flavor of fermented meat products. It was demonstrated by several authors that same strains of *Staphylococcus* were able to conduct these modifications (Masson, 1998; Leroy *et al.,* 2017).

Kocuria specie (formerly referred to as *Micrococcus*) from *Micrococcaceae* family are mentioned and associated to fermented sausages due to their potential role on color stability, due to the nitrate reduction activity of the bacteria, and producing flavor and aroma compounds through proteolysis (Tremonte *et al.,* 2007). This ubiquitous specie and highly adapted to their ecological niches (Herschend *et al.,* 2016) were identified on fermented sausages and processing environment being associated to biofilms formation (Carpentier & Chassaing, 2004). *Kocuria varians* and *Kocuria kristinae* were the species found (Fischer and Schleifer, 1980; Raghupathi *et al.,* 2016). However, the reports regarding *Kocuria* spp. frequency are scarce, since their common association to the counts of *Staphylococci* coagulase negative as a major group. Martin *et al.* (2007) referred that on the group of CNC the *Kocuria* spp. were found at levels lower than 4%. *Kocuria varians* (formerly referred to as *Micrococcus varians)* seems to improve the flavor profile in fermented sausage because of their proteolytic activity (Tremonte *et al.,* 2007), also was demonstrated its effect on putrescine degradation (Callejón *et al.,* 2015). The strain *Kocuria varians* NCC 1482 is producer of the antimicrobial peptide variacin with activity against *Enterococcus* sp., *Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum* (Pridmore *et al.,* 1996; O'Mahony *et al.,* 2001). According to Martin *et al.* (2007), although strains of *Kocuria* spp. could be employed as starter cultures in fermented sausages, it could be inappropriate in the case of Iberian dry- cured/ fermented sausages due to their long ripening time.

The mains two groups that have been studied by several authors considered of technological interest in the production of fermented meat products are the coagulase-negative Staphylococci (CNS) as well as lactic acid bacteria. Both groups can have an active role on sensory characteristics development of fermented meat products related to the lipids components. The esterases of BAL and *Staphylococcus* spp. preferentially hydrolyze substrates esterified with short chain fatty acids, however, having different specificities. The presence of esters in the volatile fraction of fermented meat products is frequent, and it has been observed that their relative abundance can be conditioned by microbial activity, sometimes by modulating medium conditions—favoring the occurrence of esterification, namely by the production of compounds involved in the reaction. The relative abundance of esters in the finished product will be the result of the balance between their generation and hydrolysis. In the formation of esters, there are authors who point out that the microbial intervention also occurs by the production of enzymes capable of catalyzing reaction, which properties are attributed to esterases, given the possibility of the enzyme also functioning in the direction of esterification. The esters, in particular the ethyl esters, give to the sausages and cheeses a fruity aromatic note.

In diverse fermented meat products with spontaneous fermentations, the yeasts and molds are commonly found in relative high number. Some of them might be related to the fermentative process, but other can be considered a spoilage microbiota. The studies about yeast biodiversity associated to the fermentation of meat sausages are scarce. Yeasts may be physiologically classified with respect to the type of energy generating process involved in sugar metabolism, namely non fermentative, facultative fermentative or obligate fermentative. Most of the yeasts identified are facultative- fermentative and are strictly aerobic. The need to access to oxygen hinders the interest of yeast in meat fermentation, once its activity became mostly limited to the surgace part of the sausages. Carbohydrates must also be available for yeast growth.

The common yeasts found on meat are *Candida*, *Rhodotorula*, *Debaryomyces*, and *Trichosporum* and this is related to environmental contamination.

Candida, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Hypopichia*, *Kluyveromyces*, *Leucosporidium*, *Pichia*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Torulopsis*, and *Yarrowia* have been the yeasts most frequently identified and described on meat and meat products. *Debaryomyces hansenii*, was the most commonly isolated yeast in dry fermented meat products (Gardini *et al.*, 2001; Cocolin *et al.,* 2006). However other species were mentioned on different survey to characterize Italian fermented sausages such as *Candida famata*, *Rhodotorula mucilaginosa, Yarrowia lipolytica, Candida zeylanoide*, *Pichia triangularis*, and *Metschnikowia pulcherrima* (Gardini *et al.,* 2001; Cocolin *et al.,* 2006). The yeast growth depends of all intrinsic and extrinsic factor related to the type of the product, environment of the factory, and the degree of smoking (Asefa *et al.,* 2006, Mendonça *et al.,* 2013). On smoked fermented sausage there are a inhibition of yeast growth on sausage surface. The growth of yeast species on sausages is mainly influenced by their oxygen requirements so their development is performed on the external surface of the product. They can form a firm and white external coat. This coat controls water loss and permits uniform dehydration of sausage. Yeasts are considered to affect sausage color and flavor due to their oxygen-scavenging and lipolytic activities (Bolumar *et al.,* 2006). The improvement of appearance, flavor and aroma is directly attributed to the ability of some yeasts species to ferment different sugars. They may also delay rancidity and further catabolize products of fermentation, such as lactate produced by lactobacilli, thereby increasing the pH and contributing to the development of less acrid and more aromatic sausages (Selgas *et al.*, 2015). Care should be taken in products which preservation depends on the reduced pH, once the catabolic activity of yeast on the lactic acid might compromise the safety. Amino acid catabolism, oxidation of lipids, esterase activity and carbohydrate catabolism are the biochemical pathways by yeasts leading to the generation of most reported volatile organic compounds on meat products (Flores *et al.*, 2004, Patrignani *et al.,* 2007). The composition of yeast mycobiota is thus an important determining factor for the quality and sensory characteristics of meat products.

The surface of fermented meat products can be colonized by molds able to grow on different environments particularly those related to meat processing characterized by lower temperatures. Fermented meat sausages with low pH and a_w will allow molds growth since they are acid tolerant, xerotolerant, and xerophilic (Filtenborg *et al.,* 1996). The genus *Penicillium* is the most widespread on fermented meat sausages and can be responsible for the surface colonization, mostly *P. nalgiovense* and to a lesser extent, *P. chrysogenum* (López-Díaz *et al.,* 2001). *Penicillium chrysogenum* and *P. nalgiovense* are able to limit growth of mycotoxigenic molds in fermented sausages (Bernaldez *et al.,* 2013). Molds have and important role in the meat products process because they can be associated to the development of specific flavors and aromas, due to their lipolytic and proteolytic activities (Sonjak *et al.,* 2011). Also, the superficial layer of mold on several fermented cured sausages is important since can create an antioxidative effect, protecting this product from development of the rancidity and keeping its color (Delgado *et al.,* 2016). This mold layer gives to particular sausages of different origins, its typical appearance and allows the development of a positive microclimate at the surface in order to prevent sticky or slimy characteristics (Visagie *et al.,* 2014).

However, the ripening conditions could favor also the growth of spoilage and mycotoxigenic molds and are one of the main indicators for shelf life prediction on meat sausages. Molds growth on dry fermented sausages has been associated with unpleasant appearance apart the potential accumulation of mycotoxins. Matos *et al.* (2007) identified on a fermented sausage several mold species related to spoilage and potential producers of mycotoxins: *Penicillium terrestres* (43.4%), *Penicillium* sp. (13.3%), *Fusarium* sp. (10%), *Aspergillus glaucus* (10%), *Aspergillus versicolor* (6.8%), *Monilia fruticola* (3.3%), *Absidia* sp. (3.3%), *Cephalosporium* sp. (3.3%), *Rhizopus stolonifer* (3.3%), and *Fusarium tricinctum* (3.3%). Mold spoilage may also occur in fermented sausages that are packaged in a modified atmosphere, reducing the shelf life and causing substantial financial losses to industry.

Strategies to Develop according to Actual Consumer Concerns

The demand by consumers for healthier food products increased from last decade. Meat products reputation became "potentially unhealthy foods" for several consumers due to their nutritional characters, namely the high fat and salt contents (Santarelli *et al.,* 2008; Klurfeld, 2015). It is generally accepted that reducing salt consumption will lead to lower blood pressure, which will in turn be beneficial for the prevention of cardiovascular diseases contributing to achieve the concept one's health (Doyle and Glass, 2010; Webster *et al.,* 2011). Increased blood pressure and hypertension are major risk factors for cardiovascular diseases and are estimated to contribute to 49% of all coronary heart disease and 62% of all stroke events (Morgan *et al.,* 2001; Aburto *et al.,* 2013). The World Health Organisation (WHO) has been supporting the development of national salt reduction strategies since 2007. Also, high fat food intake, and particularly fats with high proportion of saturated fatty acids on diet is considered a risk factor for coronary diseases. All individuals should be strongly encouraged to reduce total fat and saturated fat intake. Obesity is another public health concern related to a high intake of fatty food on diet (IARC, 2017).

Additives presence on meat products and general processed food is nowadays another matter of dissatisfaction for consumers. Additives are substances applied in small quantities during the manufacture of food to increase, restore, or enhance sensorial attributes (taste, color, texture, firmness) and shelf-life. An additive to be applied in meat products must be technologically needed and should not exceed their recommended limits of use in order to not become an hazard and be harmful to human health.

So, meat industry facing all these challenges, has been replying with reformulation of meat product recipes. Meat products are complex products in which each ingredient plays a specific role, making their reformulation a challenging task. Huge difficulties could come up since the modification of salt, fat and additives on fermented products could eventually have an impact on fermentative microbiota with sensorial attributes modification and in the microbial ecosystem, resulting in unsafe products. From a sensory perspective, salt has impact on products' texture, as well as on characteristic salty taste associated to meat products. For this reason, production of low-salt content fermented sausages must assure not only safe and stable products, but also that consumers' acceptability is not affected.

The diverse strategies used for low salt content meat products are: salt reduction, replacement of salt with a low-sodium mixture, flavor enhancers (monosodium glutamate or yeast extract), changes in the physical form of salt (Rama *et al.,* 2013; Emorine *et al.,* 2014), and improvement of salt diffusion using high isostatic pressure or ultrasound technologies (McDonnell *et al.,* 2014, Ojha *et al.,* 2016). However, to help to reduce the quantities of salt used on fermented meat products technology, only were reported strategies of reducing salt content, replacement of sodium chloride by other non-sodium salts (such as magnesium, calcium or potassium chloride) or the use of flavors enhancers on several studies describing their impact on products (Table 13.1).

Lower salty taste and less intensive color were perceived in fermented products when less salt was used (Corral *et al.,* 2013). Also, salt reduction has shown an effect on aroma produced a decrease in the aroma compounds, this included dimethyl trisulfide, 3-methyl thiophene, 2,3-butanedione, 2-nonanone and acetic acid therefore, decreased cooked, onion, butter and vinegar odors. In contrast, key aroma aldehydes (hexanal, heptanal, 2-nonenal, pentanal) increased and thus, produced the rise in herbal, grass, green odors (Corral *et al.,* 2013; Flores, 2018). When using salt replacers, those compounds usually cause unpleasant tastes (bitter,

TABLE 13.1

Salt reduction or replacement on dry fermented sausage

Products	Strategy implemented	Effects	References
	Salt reduction		
Salami	2.5% NaCl is the lower limit for acceptability of the product	At 2.25% salt content, the sausages are less firm, the typical aroma is weaker and the yield lower than with higher salt contents.	Petaja *et al.*, 1985
ChouriçoPreto and *PaioPreto* (blood fermented sausages)	Salt reduction from 6% to 3%	Biogenic amines levels increased. Texture profile analysis revealed lower resilience and cohesiveness in low-salt products, although no textural changes were observed by the sensory panel.	Laranjo et al., 2017
Catalão and *Salsichão*	Salt reduction from 6% to 3%	Salt content had a major effect on microbiological counts, although not compromising the products' safety. Higher amounts of histamine, tyramine and cadaverine, in products with 3% salt. Low-salt products revealed to have a decrease in hardness and chewiness, along with an increase in adhesiveness values. Sensory evaluations revealed that despite the less intense aroma, products with 3% salt, had a more balanced salt perception.	Laranjo *et al.,* 2016
	Salt replacement		
Fermented sausages	KCl (0-60%), potassium lactate (0-100%) and glycine (0-100%)	Bitterness is the only limiting factor in KCl substitutions. It is recommended KCl<40%. High K-lactate substitution levels (> 40%) present problems in texture, color and flavor. Delay in pH decrease. NaCl substitution with glycine could be acceptable at a 40% level.	Gou *et al.,* 1996
Chorizos	50% reduction of NaCl using potassium, magnesium and calcium ascorbates	Lower consistency since the chloride ions are replaced with ascorbate ions and do not effectively react with myofilaments. Sensorial acceptability was lower mainly due to the lower salty taste.	Gimeno *et al.,* 1998, 2000
Slow fermented sausages	control (2.7% NaCl, S), 16% salt reduced (2.26% NaCl, RS) and 16% replaced by KCl (2.26% NaCl and 0.43% KCl, RSK).	Small salt reduction (16%) (RS) affected sausage quality with a reduction in the acceptance of aroma, taste, juiciness and overall quality. Salt reduction affected aroma due to the reduction of sulfur and acid and increase of aldehyde compounds. The substitution by KCl (RSK) produced the same acceptability by consumers as for high salt (S) treatment except for the aroma that was not improved by KCl addition.	Corral *et al.,* 2013

(*Continued*)

TABLE 13.1 (Cont.)

Products	Strategy implemented	Effects	References
Salami	Lower Na concentration and salt replacers (five replacement levels of NaCl by KCl mixed with $CaCl_2$)	Were observed differences in pH and Aw (water activity) due to NaCl reduction. Salt replacement mixtures of KCl/$CaCl_2$ in salamis did not affect this process technologically (slicing, appearance and texture), and the decrease in Na content was approximately 55 %. The production of low sodium salamis using salt replacers (KCl and $CaCl_2$) or salamis without replacers with a value higher than 1 % of NaCl can be used effectively without compromising major sensorial attributes.	Almeida *et al.,* 2013
Brazilian dry fermented sausages	control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by $CaCl_2$ (1.25% NaCl and 1.25% $CaCl_2$, F3), and 50% replaced by KCl and $CaCl_2$ (1.25% NaCl), 0.625% KCl and 0.625% (CaCl2, F4).	Neither 50% reduction nor replacement of NaCl by KCI affected the volatile compounds produced during the manufacturing process, and both increased the volatile compounds from carbohydrate fermentation and amino acid degradation during storage. The $CaCl_2$ increased the generation of volatiles from lipid oxidation during processing and storage of dry fermented sausages, thus indicating that the replacement of 25 and 50% NaCl by $CaCl_2$ is not a viable alternative.	Santos et al., 2015
	Salt reduction or replaced + flavor enhancer		
Fermented sausage	Fermented sausages with 25% or 50% of their NaCl replaced by KCl and supplemented with 1% or 2% concentrations of yeast extract (*Saccharomyces cerevisiae*) were produced	The sensory quality of the fermented sausages with a 50% replacement was poor compared with the full-salt control samples. The use of yeast extract at a 2% concentration increased volatile compounds that arose from amino acids and carbohydrate catabolism. These compounds contributed to the suppression of the sensory-quality defects caused by the KCl introduction,	Campagnol *et al.,* 2011
Dry fermented sausage	Inoculation of a *Debaryomyces hansenii* strain in dry fermented sausages with reduced 17-20% salt contents	*Debaryomyces hansenii* compensated partially the effects produced by salt reduction. The yeast inoculation on salt reduced sausages (RS + Y) was accepted better than uninoculated salt reduced sausages.	Corral *et al.*, 2014

metallic, astringent) and could also affect texture properties, resulting in consumers rejection of the product (Almeida *et al.*, 2018).

Fat reduction on fermented sausages has been object of several studies (Table 13.2).

The strategies related with fat content could be the single reduction of fat on formulations, the substitution of fat with fat replacers or the modification of fat with a different fatty acid profile

TABLE 13.2

Fat reduction and replacement of dry fermented sausages

Products	Strategy implemented	Effects	References
	Fat reduction		
Dry fermented sausages	Dry fermented sausages with different fat contents were produced (10%, 20% and 30%).	Fat reduction decreased the generation of lipid derived volatile compounds during processing while those generated from bacterial metabolism increased, although only at the first stages of processing. The consumers preference was for the high and medium fat sausages related to the aroma compounds	Olivares *et al.,* 2011
	Fat reduction and replacement		
Dry fermented sausages	fat levels (30, 20 and 10%) and two levels (0 and 20%) of pork back fat replacing olive oil.	Low-fat fermented sausages with or without olive oil, have an unacceptable appearance, due to intensively wrinkled surfaces and the development of case hardening. The reduction of fat level increases the weight losses, the hardness and firmness of sausages and makes them darker and redder. The replacement of 20% pork backfat with olive oil does not affect the weight losses but makes the sausages lighter in color and more yellow.	Muguerza *et al.,* 2002
Salami	Four 'salami' formulations, with 15% total fat, were prepared, in which 0, 33.5, 50 and 66.5% of pork backfat was substituted with extra-virgin olive oil.	The difference between samples with and without extra-virgin olive oil was a piquant note detected in the oil-added 'salami'. The formulation with 5% olive oil, corresponding to 33.3% substitution of pork backfat with olive oil, was judged best of all formulations. The results show that it is possible to produce this type of product, of good taste, similar to the traditional one, but with healthier features.	Severini *et al.,* 2003
Dry fermented sausages	50% and 25% fat reduction The 25% batch was supplemented with different amounts of soluble dietetic fiber (inulin) as both a powder and in aqueous solution.	Results obtained indicated an overall improvement in the sensory properties due to a softer texture and tenderness, springiness and adhesiveness similar to the conventional high fat sausage.	Mendoza *et al.,* 2001
Chorizo de Pamplona	Dry fermented sausages with a partial substitution of 15, 20 and 25% of pork backfat by pre-emulsified soy oil were prepared	No increase in oxidation was detected. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) decreased in the modified formulations, to 32.81 and 42.09 g/100 g of total fatty acid in the 25% replacement products, respectively. Polyunsaturated fatty acids (PUFA) increased from 15.22 (control) to 23.96 g/100 g of total fatty acid (25% product) due to the significant increase in linoleic and a-linolenic acids when soy oil was	Muguerza, et al., 2003

(*Continued*)

TABLE 13.2 (Cont.)

Products	Strategy implemented	Effects	References
		added. Hardness and springiness did not show significant differences among products. Sensory evaluation of most of the modified sausages did not show significant differences with regard to the control.	
Chorizo de Pamplona	Linseed oil gelled carrageenan emulsion as a replacer of animal fat in fermented sausages Replacement were 26.3% (SUB1), 32.8% (SUB2) and 39.5% (SUB3) α-linolenicacid (ALA) content increased up to 1.81, 2.19 and2.39 g/100 g (SUB1, SUB2, and SUB3 products) as compared to the Control (0.35 g/100 g)	Dry fermented sausages enriched in α- linolenic acid could be successfully elaborated with an adequate technological process by the incorporation of a carrageenan gelled emulsion prepared with linseed oil. Improvement of the lipid profile of reformulated products by increment in omega-3. The reformulation process did not cause oxidation problems, and no perceptible differences were reported for taste and juiciness as compared to a traditional product.	Alejandre *et al.,* 2016
Chorizo de Pamplona	Fish oil extracts rich in *n*-3 fatty acids were manufactured in order to obtain functional products (5.3 g kg^{-1} and 1.07 g kg^{-1} of a fish oil extract (omega-3 700 Solgar Vitamins Ltd, Newcastle, UK) were added)	The addition of fish oil to dry fermented sausages significantly decreased the *n*-6/*n*-3 ratio leading to values closer to that considered optimal. However, samples with the highest oil extract addition showed a great increase of TBARS values indicating that oxidation could be a problem during storage.	Muguerza *et al.,* 2004
Dutch style fermented sausages	10%, 15% and 20% of pork backfat by flaxseed oil and canola oil, pre-emulsified with soy protein isolate. The 15% and 20% substitution were also reached by adding encapsulated flaxseed oil and encapsulated fish oil and by adding flaxseed oil, pre-emulsified with sodium caseinate	The addition of canola oil and encapsulated flaxseed oil resulted in a comparable shelf life as the control in terms of lipid oxidation. In the samples with addition of pre-emulsified flaxseed oil, especially with sodium caseinate, lipid oxidation clearly increased during storage. Physical and sensory analysis showed that the sausages with encapsulated fish oil and flaxseed oil resembled the control most.	Pelser *et al.,* 2007
Chorizo	Konjac-based oil bulking system. Reformulation of sausage with a oil combination (olive, linseed and fish oils) stabilized in a konjac matrix.	The reformulation process produced a decrease in all sensorial parameters and texture, compared with the control sample. This strategy allows an important fat and energy value reduction.	Jiménez-Colmenero *et al.*, 2013

(fat based fat replacers) (Paglarini *et al.,* 2018). It is well known that the animal fat used in the elaboration of fermented dry sausage contains a higher proportion of saturated fatty acids (SFAs) than polyunsaturated fatty acids (PUFAs). When it was tried to reduce the fat content, the low fat sausages become hard and dry in texture due to the high weight losses during drying and due to the lack of the juiciness provided by the fat. They also have an unacceptable appearance because of wrinkled surfaces (Muguerza *et al.,* 2002). However, the higher impact of lowering fat content is related to sausages flavor (Flores, 2018). In dry fermented sausages fat reduction produces a decrease in the generation of lipid derived volatile compounds (Olivares *et al.,* 2011). In terms of key aroma compounds, fat reduction decreased the generation of lipid derived volatile compounds during processing namely hexanal, (E)-2-nonenal, (E,E)-2,4-nonadienal, ethyl butanoate and 1-octen-3-ol and thus, minimized the green, medicinal, tallowy, fruity, and mushroom notes that contributes to the characteristic aroma of fermented sausages. Conversely, the aroma compounds 2-methylpropanal, 3-methylbutanal, 2-octanone, methional, methanethiol and acetic, butanoic, and octanoic acids rise intensifying the green, herbal, floral, cooked-potato, rotten, vinegar and cheesy odors (Olivares *et al.,* 2011; Flores, 2018). These changes in aroma compounds on low fat dry fermented sausages affect consumer's choices with a clearly preference for high fat sausages (Olivares *et al.,* 2011).

The replacement of pork back fat, the main source of saturated fat, using oils (fat based fat replacers) in their liquid form such as inulin, olive oil, fish oil, linseed oil, soy oil, was tried by several authors improving the lipid profile and lowering fat content on fermented sausages. Most of these studies were performed using oil in water emulsion (O/W) systems, useful to incorporate components in the lipid phase, (*e.g.*, ω-3 unsaturated fatty acids or antioxidants) and improving the fatty acid profile in dry fermented sausages, however the stabilization of these oils is needed to preserve the textural properties of the products. So, the development of emulsion gels with functional ingredients (oils richer in ω-3 unsaturated fatty acids) is an innovative strategy (Paglarini *et al.,* 2018) to apply in dry fermented sausages. Fat reduction is achieved in this case by adding ingredients to lower the fat density that are selected for their low calorie content and their ability to impart desired characteristics to the meat product, namely texture hardness and juiciness, by addition of carbohydrate or protein based fat replacers. One such ingredient is konjac (glucomannan)-based fat analogs. These form gels which, when combined with other ingredients (starch, carrageenates, gellan gum), can be used as 'fat analogs' in the formulation of reduced/low-fat meat products. Incorporation of oils in a gel-like matrix to form an oil bulking system, in which this new ingredient acts as an animal fat replacer, could offer new possibilities. In this regard, oils in a konjac matrix have been used to improve fat content in dry fermented sausage however the sensorial characteristics were far from dry fermented sausages with pork fat, indicating that the reformulation must be explored in order to overcome this inconvenience (Jiménez-Colmenero *et al.,* 2013).

Additives became a word that should not be pronounced for consumers. This negative perception worsened by the report associating meat products to colon cancer by IARC/WHO. However, as was described before some additives on fermented meat products have important technological roles. One of the main chemical concerns we have actually associated to fermented meat products are the potential risk that nitrite and nitrate represent related with nitrosamines formation (Andree *et al.,* 2010; Alahakoon *et al.,* 2015). Nitrate and nitrite role on fermented sausages is essentially related with color development and its replacement is very difficult. However, from the report of International Agency for Research on Cancer (IARC) the N-nitroso-compounds (NOC), including *N*-nitrosamines and *N*-nitrosamides, are among the factors that have stronger evidence of having responsibilities in colorectal cancer. N-nitroso-compounds are formed when nitrosating agents encounter *N*-nitrosatable amino compounds, their nature and concentration, the reaction medium, pH value, and further modifying factors, including the presence of catalysts or inhibitors of *N*-nitrosation will influence nitrosation rate. De Mey *et al.* (2014) did not observe any correlation between N-nitrosamines, residual nitrite and biogenic amines in commercial dry fermented sausages.

Several strategies have been studied to reduce the use of nitrate and nitrite (NOx-), namely the use of sources of NOx-, other than the chemical additive (Fraqueza *et al.,* 2018). Interesting results were obtained replacing chemical nitrates and nitrites by plant extracts richer in nitrate, particularly celery (Sebranek *et al.,* 2012). Celery have an atypical aroma that might pose limitations on use in certain fermented meat products. However, there are other aromatic plants that, due to its high nitrate level (Santamaria, 2006) and tradition of use could be used on products. *Caspium annunn* varieties present high levels of nitrate (300–1100 mg/100g) (Guil-Guerrero *et al.*, 2006). They are commonly used in the seasoning of dry fermented sausages and those with higher nitrate level and assuring a safe level, could be an alternative source of nitrate. Additionally, the seasoning period of meat, with a low pH and ethanol presence from wine (Gago *et al.,* 2007) and the presence of nitrate reducing coagulase negative cocci (GCC+) may contribute to nitrite rising level in the first phases of the processing, controlling the growth of hazardous pathogens while water activity is high and LAB are still beginning to dominate the microbiota of the product.

Other promising strategy that has been studied is the use of selected microorganisms (starter cultures) with specific characteristics to allow a suitable development and stabilization of the color and preventing pathogens growth.

The coagulase negative staphylococci (CNS) has been used for their ability to modulate the amount of NOC, through the reduction of nitrate to nitrite. *S. xylosus* is one of the most promising, due to their metabolic characteristics that remain underexplored. *S. xylosus* has several oxidative stress resistance mechanisms, such as modulation of the expression of several genes involved in iron homeostasis and antioxidant defense (Vermassen et al., 2014). Moreover, was demonstrated by Morita *et al.* (1998) in a fermented product *salami*, the conversion of metmyoglobin to nitrosomyoglobin by *S. xylosus* without addition of nitrate or nitrite. Li *et al.* (2013, 2016) notice the same in a raw meat batter. The mechanisms of that formation of NO color derivatives without addition of nitrate or nitrite was recently described and involves the ability of NO synthase (NOS) of certain strains of *S. xylosus* to produce NO, that reacts directly with the myoglobin (Ras *et al.,* 2018). According to these authors, NOS-dependent NO production in *S. xylosus* needs to be further characterized and optimized in meat products as a potential alternative to NOx-. Besides de potential use of these NOS production strains to the formation of the cured color, the NO formed by CNS can contribute to the inhibition of *Clostridium* spp., once it is the compound, usually available for the inhibition through its interaction with iron-sulfur proteins, such as ferredoxin and ferrodoxin-pyruvate oxiredutase, with a decrease of ATP and pyruvate accumulation (Hospital *et al.,* 2016).

Quality Control of Fermented Meat Products

The word quality is commonly used in diverse contexts and is often misused, without specifying any attributes or requirements of that supposed ‘quality’. Quality attributes of any product, including foodstuffs and related services, may be framed under different perspectives, depending on the stakeholder perspective. From a point of view of the food consumer, the sensorial quality will be, virtually, the most valued attribute and that leads to the preference of the brand and customer loyalty. The consumer assumes the food safety as granted, or when he has doubtful, he avoids acquiring the foodstuff, looking for other brands or substitute products. Some consumers, particularly concerned about the nutritious balance of their food, look food quality from a nutritional perspective, searching for healthier or allegedly healthier foods. These consumers currently represent a potential niche market for low salt, low fat or foods with high ratios of unsaturated fats. From the point of view of the manufacturer, quality could be seen through the same consumer perspective, as it will ensure the selling of their products, when are attained consumer preferences. In addition to consumer satisfaction, the manufacturer is concerned with the technological quality. This regards the suitability of raw materials to transform and the company’s installed technical capacity to manufacture food products according to safety and legal requirement. Other indirect aspects might be considered in this generic assessment of quality, namely ethical issues associated with animal welfare or environmental protection, social responsibility of the business in a concept named total quality (Sijtsema *et al.,* 2002). The importance of quality control is well recognized as a management tool to assure the success of the business, by protecting the brand through the consumer protection from potential hazards and from unexpected fails of quality or not compliance with quality specifications (Figure 13.6).

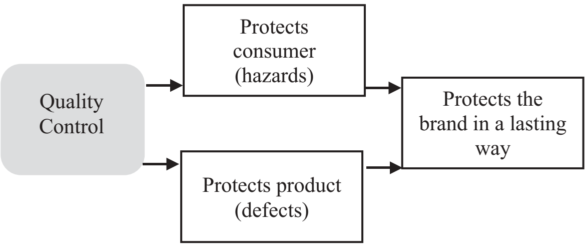
Quality Control
Protects consumer (hazards)
Protects product (defects)
Protects the brand in a lasting way

FIGURE 13.6 Relationship between quality control and brand protection.

From a more pragmatic perspective, and according to the quality management systems policy, quality is defined as the fulfilment, or satisfaction, of the customers' requirements. These requirements are usually grouped into two categories: implicit and explicit. The implicit requirements are those that are expected in the product, and there is no need to clarify them in a business contract. According to the ISO 9001 (2015), implicit requirements are those arising from the framework of the production or from the law. For example, when establishing the quality requirements of a fermented sausage for the manufacturer and customer, it is not necessary to state that the product should not contain *Salmonella* spp. in 25 g or should not include phosphate levels above 0.5 g/kg as these requirements are established by law, in UE, according to Regulation EC, 1441 (2007) and Regulation EC 1129 (2011), respectively. Explicit requirements are specifications required by the customer, negotiated with the manufacturer who accepted them, and may include formulation specifications, sensory or nutritional characteristics, presentation or price specifications. Continuing with the example of the above-mentioned fermented sausage, the explicit requirements could be a specific pH, fat content, or a certain extension of products ripening, to gain a superior aroma. Regarding the quality control framework for fermented meat products, the manufacturer must accomplish three different approaches (Table 13.3). In terms of planning quality control, these three approaches have different purposes, they must be merged to optimize the safety and quality control tasks, and save time and work power.

Hazard Analysis and Critical Control Points (HACCP) is recognized as the reference method to Food safety management in all the food chain, with a universal application worldwide since its adoption by FAO/WHO *Codex Alimentarius* about thirty years ago. This standard method had experienced several revisions, namely in organizing the food safety activities in prerequisite programs and HACCP plans in 1997. The efficacy of the HACCP depends on the correct application as a whole,

TABLE 13.3

Compatibility between HACCP and other quality control systems

HACCP	Legal Conformity	Quality control
Hazard Analysis	Established obligations	Defining the requisites: Costumer requirements/absence of defects
Critical Control Points (CCP)	Legal ControlPoint (LCP)	
Critical limits	Previewd in theLaw	Conformity of requisites
PCC Monitorization	LCP Monitorization	Inspect; monitorise, test
Corrective Actions	Corrective Actions	Reprocessing or withraw
Verification	Verification	
Documentation	Documentation	Documentation

and if any part has a fail, it might have serious consequences for the safety of the product. In the preparation of the HACCP plans, still all the principles are important, the correct and adequate hazard identification and analysis previewed in principle one, is of upmost importance for the chain of thought and success of the whole planning. Conducting a hazard analysis requires the identification of the potential hazards associated with all stages of the process, from raw materials to the final consumer (Codex Alimentarius, 2009). In the processing of fermented meat products *Campylobacter, Salmonella, Escherichia coli* O157:H7, and other enterohemorrhagic *E. coli* (EHEC), *Listeria monocytogenes*, and *Clostridium* toxins or from other *Bacillaceae* have been the predominant biological hazards associated to these products and involved in human foodborne diseases (MMWR, 2018; EFSA, 2018). Dry fermented meat products, since most of them are ready to eat meat products, should be regarded as a potential vehicle of these microorganisms according to the main raw materials involved in their production. Also, dry fermented meat products suffer handling for slicing and other operations that might allow product's recontamination with *L. monocytogenes.* These potential biological hazards are recognized on fermented meat products but other safety hazards (chemical) are on the order of the day such as nitrosamines, biogenic amines (BAs), polycyclic aromatic hydrocarbons (PAHs), phenols and formaldehyde, heavy metals, biocides (desinfectants, pesticides, antibiotics residues), mycotoxins (Fraqueza and Barreto, 2015; Pussa, 2015; Reig and Toldra, 2015; Ledesma *et al.,* 2016; Alves *et al.,* 2017).

The start point of a safety control system applied to fermented meat products will be the safety of raw materials entering on process (Fraqueza, 2015), the implementation of procedures regarding selection and control of suppliers, ingredients traceability, hygiene and maintenance programs, residues management, pest control are preventive measures necessary to prevent the entrance of several hazards in the process of meat fermented products. All these preventive measures are the prerequirements that are the foundations to a flexible model of a food safety management system adapted to all kind of industries (micro, small or large). These preventive measures associated with the HACCP method will give us assurance for fermented meat products safety (Fraqueza, 2015).

The identification of potential hazards based on scientific literature, epidemiologic evidences or historic hazards assessment of the industry will allow to make a hazards analysis and estimate the risk (occurrence/severity) of each hazards according to an a approach previously defined (FAO, 1998, FAO/WHO, 2009).

The identification of critical control point in the process of fermented meat products should be done according to the method of the *Decision Tree* reasoning. However, should be emphasized that fermentation, drying and smoking are the main critical control points associated to good manufacturing practices (Fraqueza, 2015). The percentage of product weight loss and its relationship with final product aw and pH, the ripening time and the control of ripening room temperature and relative humidity are parameters to be monitored. When the natural drying is the practice, the percentage of product weight loss of the product should be recorded.

The HACCP plan should be constructed on reasonable actions of monitoring, with defined parameters criteria associated to processing conditions that will give a particular final product. This plan should preconize corrective action of potential fails that could occur. The verification of the implemented HACCP system is based on evidences resulting from all the records performed from the prerequirements implemented and all actions taken regarding the control of critical points of the process. Also, for verification of the safety management system implemented, an analytical plan can be structured including sampling frequency, microbial and chemical parameters, defined criteria and definition of actions responsibility. Microbial analysis of fermented meat sausages, namely those analyses related to the quantification of certain microbial groups that are indicators of spoilage and hygiene level, and those for detection/quantification of pathogens, offer information on whether the system is reaching the targets. However, an efficient system must establish the real significance of each parameter analyzed in order to avoid wasting time and money. All the results obtained from verification actions will be considered as inputs for the revision of the safety management system implemented according to a cycle of continuous improvement of safety.

The brainstorming and methodology assigned for safety could be extended also for quality. The construction of a quality plan with preventive measures should be implemented in order to avoid fails of quality. There are particular steps of the process that must be controlled in order to achieve the level of quality defined to the fermented meat product avoiding fails

In Table 13.4 are presented several examples of defects that can occur in fermented meat products, associated to different steps of processing. Generally, when working with a good quality raw materials—using adequate procedure for supplier selection and control and well defined purchasing specifications, several potential defects are easily avoided (Escanciano and Santos-Vijande, 2014). The adequate color formation is dependent on the use of the correct amount of nitrite, and on the conditions to the adequate formation of nitrosomyoglobin, namely the reduction conditions that can be favored by using ascorbic or erythrobic acid, as well as the pH (Sebranek and Bacus, 2007).

The correctness (good manufacturing practices) in the operations of stuffing and smoking might contribute to avoid a considerable number of defect on the texture, internal and superficial aspect and cohesion of the sausages. It is probably the drying phase that is more prone to the occurrence of defects. The kinetic and extension of moisture loss might have serious consequences on the quality of the fermented sausage. When equipment to control temperature and RH is not used, one of the frequent problems found is the case hardening, due to an excessive loss of moisture in the beginning of the process, resulting in an interior that cannot dry, and then several other problems occur, namely those associated to the multiplication of anaerobic microorganisms (Baracco *et al.*, 1990). Still during the drying, but also in the storage, the abnormal multiplication of aerobic microorganisms in the surface are one of the biggest challenges for manufactures. When fermented meat products are finished with molds, since there are guarantees of the purity and adequacy of the culture, it is not expected that other surface microorganism growth, once the competition with the inoculated mold at massive quantities would be difficult (Delgado *et al.,* 2016). In meat products that are not finished with molds, the growth of these microorganisms is a defect that must be avoided (Pitt and Hocking, 2009). Additionally, bacteria or yeast can growth in the surface, resulting in visible growth that depreciates the product (Walz *et al.,* 2018). Lipid oxidation is fundamental to an adequate development of the characteristic flavor of dry fermented meat products, however, if these reactions are too extensive the flavor might evolve to rancid notes, that are depreciative. The boundary between the required oxidation and the excessive—rancid, is not easy to establish, thus the acceptance of the consumer should be known in order to provide the more adequate product for their requirements (Kumar *et al.,* 2015).

According to Frank Moerman (2018), food quality refers to the quality specifications of a food. So, looking to a fermented meat product we can recognize that there are three groups of quality specifications for final products: microbial, chemical and sensorial. To obtain the expect results, several aspects should be controlled with preventive measures during all the processing. In Table 13.5 are shown several examples of actions that could be used in the fermented sausages control, having in consideration the application of HACCP methodology, other than HACCP legal obligations and costumer requirements/absence of defects.

Besides all the control measures made during the processing, in the final product it can be recommended to analyze those parameters that are mandatory by law with the application of an HACCP plan (verification actions with a analytical plan as referred before). Microbial criteria might include the absence of *Salmonella* spp. in 25 g counts of *L. monocytogenes* below 100 UFC/g if the product have a high aw and/or pH. Presence of toxin from *Staphylococcus aureus* might be a requirement from some customers. The count of LAB and SCN might be useful to confirm the adaptation and dominance of that microflora. Common counts of Total Viable Microflora at 30ºC or at 10ºC is useless, once the beneficial fermentative microflora is present in the products.

Determination of aw and pH are important in the final product to verify that the drying and fermentation was enough to confer preservation parameters to the product, particularly if they are to be market at room temperature If water activity is considered alone, it should be below 0.91. When combined with a pH < 5.0, the aw might be slightly higher (< 0.95). In products with a deep fermentation, and large amount of acid produced, the pH ≤4.5 alone is enough to assure the preservation of the fermented products at room temperature. When these parameters do not allow, products must be maintained in refrigeration.

TABLE 13.4

Examples of quality fails and defects associated to the different steps of fermented meat products processing

Process steps	Quality Fails/ defects	Cause	Control measures	Critical limits	Monitoring actions
Reception	Bad connection of the pieces on final product Soft or oily final product Abnormal colors of final product	Meat and ingredients quality Fat quality; excessive amount of unsaturated fatty acids High level of microbial contamination on meat	Ingredients Specifications/suppliers selection and control Correct temperature of delivery (<7 ºC)	pH of lean meat ≤5.8 and ≥ 5,6) Temperature <7ºC	Check documents from Suppliers, measure meat pH, Temperature, visual inspection
Weighting ingredients and additive	Fade or intense red color	Lack or excess of nitrate and nitrate	Checklist of additives weighed and added Calibration of scales	The defined in the formula	Adequate weighting control. Double checking the weighing of additives
Resting period	Abnormal color of the final product Bad connection of the pieces	Incorrect color formation due to the dynamics in nitrite and nitric oxide; eventual lack of reductor additives; insufficient distribution of colored ingredients (paprika) or colorants Incorrect distribution of salt	Temperature of refrigeration room, time of resting, Relative humidity; Check the correct amount of reductor additives (ascorbic acid or other) Mixing if necessary	Temperature< 7ºC %RH ≈ 90% 24h-48H	Measurement of room temperature, relative humidity and resting time; Visual inspection of the color of the paste to lookout for abnormal colorations
Stuffing	Final product soft and hollow; Separation of the casing or wrinkled casing	Soft Stuffing	Good manufacturing practices	Absence of holes	Visual inspection at defined regularity
Fermentation	Bad connection of the masses on final product Sour taste and too fermented aroma Holes	Rapid or excessive acidification Heterofermentative strains are involved in the fermentation	Temperature, time, relative humidity, Amount of sugar added pH Check the identity of the strains use din starter cultures or change the supplier	Temperature between 15 to 22ºC slow fermentation, 22-26ºC for fast fermentation; %RH≈90% Amount of carbohydrates defined in the formula (≤1%) Absence of gas pH 4.8–6.0, according to particular traditional fermented sausage	Measurement of temperature and pH Measure temperature, humidity, and air velocity; Visual inspection

(*Continued*)

TABLE 13.4 (Cont.)

Process steps	Quality Fails/ defects	Cause	Control measures	Critical limits	Monitoring actions
Smoking	Superficial Color not stable Fade or excessive smoke aroma Fungi in final products surface	Cooling in the exit of smoking amount/ time of smoking Weak smoking	Adequate amounts of smoke, control de RH while smoking; very dry chambers might hinder the smoke to adhere to the product Smoke correctly (if the case)	RH not below 95% during the smoking	Visual inspection at the end of smoking
Drying	Case hardening Soft or hard sausages Abnormal colors, holes and bad cohesion might be associated to any of the upper referred defects Oxidation	Drying too fast at the beginning Insufficient or excessive drying No catalase activity	Temperature, RH and air velocity should be controlled pH and aw control weight loss Add antioxidant ingredients or additives; review the survival of CNC	12-16°C 70-80% RH Air velocity=0.05 ms^{-1} aw =0.85–0.90 % of weight loss according to industry definition (30-40%)	Visual inspection for defects detection Measure temperature, humidity, and air velocity; Measure aw and sausages weight loss
Packaging and Storage	Undesired mold growth Rancidity Saline or phosphate precipitates Bloated package	Contaminated environment, storage in moisten environments; access to oxygen, and for oxidation other catalytic factors, as light Temperature and RH variations before packaging (the cause could be upstream, related to meat quality and drying kinetic) Multiplication of gas producing microorganisms	Store without oxygen and avoid light exposure; use antifungal additives in the casings; Avoid variations in temperature and RH Check the identity of the strains use din starter cultures	100% Package integrity; correct MAP sealing 100%, gas mixture without oxygen temperature< 7°C, 70-80% relative humidity, and air velocity ~0.05 m/s Absence of bloating	Visual inspection; MAP sealing bags assessement Measure % Oxygen in the package Measure temperature, RH% and time

Chemical analysis should include those parameters necessary to accomplish the nutritional labeling (protein, total fat, unsaturated fat, salt and sugar) and to verify that the chemical hazards are below the recommended levels, namely for polycyclic aromatic hydrocarbons and food additives. Both microbial and chemical parameters are used mainly as verification of safety and legal compliance. The sensory attributes are the more important to meet consumer expectations, and to keep the brand loyalty. Several approaches might be used. Some industries have sensory analysis panels to perform analytical tests to follow the sensorial quality of the product. These tests are usually very useful, but they are expensive, due to the need of having selected and trained panel in the industry, or outsource that work, that is usually expensive. Working with consumers is also an interesting approach, once, independently of the characteristics of the product, one might know directly how will be the acceptance of the product that is the final objective.

TABLE 13.5

Examples of monitoring or verification actions to include in the quality control of fermented meat products

Activity	Control	Requisites
Raw materials		
Meat (cuts or trimmings)	Compliance with specifications	As defined with the supplier
	Temperature in reception	< 7ºC
	General hygiene	Absence of abnormal smell or color
	pH	pH ≤ 5.8
Casings	Compliance with specifications	As defined with the supplier
Other ingredients	Compliance with specifications	As defined with the supplier
Processing		
Fermentation	Temperature	More adequate for the desired acidification kinetic—15 to 22ºC slow fermentation, 22-26ºC for fast fermentation;
	pH	According to the preservation characteristics (see below)
Smoking	Temperature chamber	< 20-30ºC
	Temperature of burning wood	Avoid smoking with live fire. wood burning < 500ºC
Drying	Temperature	12-16ºC
	Relative humidity	70-80% usually HR values should be about 5% lower than the aw in that drying phase
	Product weight loss/aw	The aw required might be 0.91 (if the pH is higher than 5.0) or 0.95 (if the pH is lower than 5.0) Weight loss might be used to assess the level of drying—usually 25 to 40% of weight is loss
Final product		
Physico-chemical analysis	Aw and pH (for products market without refrigeration)	0.91 if pH>5.0 0.95 if pH≤5.0 If pH<4.5 no need to reduce aw
	Nutritional composition	Protein, carbohydrates, fat and saturated fat, salt
	Additives (for verification purposes)	Legal limits
	Benzo(a)pyrene g) Sum of benzo-(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	≤ 2 µg/k ≤ 12 µg/kg
Microbial analysis	Absence or controlled growth od defined hazards (for verification purposes)	As defined in HACCP plan

(*Continued*)

TABLE 13.5 (Cont.)

Activity	Control	Requisites
	Salmonella spp.	Absent in 25g
	Listeriamonocytogens	Depending on the aw of the product
Sensory analysis	Molds or flour in the surface (for products not finished with mold)	Absent
	Mold finishing (for products finished with mold)	Uniform and characteristic
	Texture and aroma	As defined for the produce
	Detectable defects (holes, abnormal coloration, rancid aroma, abnormal sour taste, …	absent

Final Remarks

Fermented meat products have a long history of consumer valorization and preference, related with a deep cultural rooting in the gastronomy of several worldwide regions and food safety assurance. Nowadays the industry of fermented meat products faces several threats, mostly related with potential hazards or concerns they might represent to human health. The concerns are due to eventual high amount of fat, largely saturated fat, and salt, as well as the potential relationship that certain chemical compounds have with colon cancer, related to the use of nitrite and to smoking processing step. Due to the enormous amount of information that population have access in the internet, most of which is not validated, the communication with the consumer is probably one of the biggest challenges that fermented meat products industry have to face. In that communication it is necessary to inform correctly the consumer to understand that these products, still having some potential risk, might be included in a healthy diet.

Nonetheless these threats, fermented meat products have several characteristics that give them some advantages and opportunities. The uniqueness of the sensory characteristics of high quality products is probably the most important aspect to consider. Also, the context of fermented meat products production, particularly those from mountain and agriculture regions, is an inheritance to its reputation. These products are frequently produced from pigs of breeds that were condemned to extinction in the last decades, due to the strong selectivity of the pigs for high productivity of lean carcasses. The use of these breeds for processing was responsible for the maintenance, or even growth of several autochthonous pig breeds populations worldwide. In these contexts of production, the animal welfare and the low energetic input of fermented products manufacturing is also an opportunity to communicate with the informed urban consumer.

Using adequately the knowledge gathered by years of research, the manufacturing of fermented meat products has challenges to became healthier and environmental friendly. The trend of reducing or eliminating chemical additives, particularly the problematic nitrite, has been the target of a considerable amount of robust research, that allows us nowadays to apply alternatives in certain fermented products. However, it is in the research related to the fermentative microbiota that the advances are more promising, both by the vast understanding of the antagonistic activity of bioprotective LAB, allowing to select strains able to inhibit the main pathogens of concern in meat products, as well as the contribution to the sensory characteristics, particularly by *Staphylococcus* spp. strains, through several metabolic activities that are known since the last decades, but also by the ability to produce nitric oxide that some strains demonstrate in recent research, that opens great opportunities to eliminate nitrite from the manufacturing and return to a more natural product, made with the ancestral knowledge, and now supported by scientific evidences.

With the knowledge available on the phenomena involving the fermented meat products manufacturing, an adequate system of quality control and food safety, implemented through the numerous tools available for that effect, is determinant to guarantee the high levels of food safety that are demanded by the consumer and compulsory by law, to improve the nutritional profile and to maintain or improve the exceptional sensory characteristics from which these products are recognized

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Fermented Fish Products

Amanda L.A. Jamas
Universidade Federal Fluminense – UFF, Rio de Janeiro, Brazil

Bruno V.V. Pinto
Centro Universitário de Valença – UniFaa, Rio de Janeiro, Brazil

Amanda F.C. Estanech, Elizete Amorim, Pedro Paulo O. Silva, Romulo C. Valadão and Gesilene M. de Oliveira
Universidade Federal Rural do Rio de Janeiro – UFRRJ, Rio de Janeiro, Brazil

1 Introduction

Fermentation is one of the oldest traditional methods used in the preservation of fish and other foods. In addition to guaranteeing its perfect conservation, it also maintains the nutritional quality, highlighting the bioavailability of proteins (amino acids and peptides) and high digestibility, such as the sensory quality of flavor, aroma, texture, and color. Cured fish products (dried, salted, brined, fermented, and smoked) accounted for 12% of processing, given the 151.2 tons (88% of the 171 million tons) of fish destined for direct human consumption in the world in the year 2016 (FAO, 2018). Usually underutilized and smaller fish are the most used for processing, giving a better use to fishing resources without economic relevance, providing the population with a complete and balanced nutritional diet.

Fish products obtained by fermentation in several countries of the world have been part of the nutritional diet of the most needy populations serving a regional and local market. Typical products (fish and crustaceans, anchovy, marinated, and salted fish and sauces) from the fermentation of fish are marketed in Southeast Asia, Northeast India, Africa, Europe, and South America, and countries such as Argentina, Brazil, Chile, and Peru produce semi-preserves of anchovies and sardines on a small commercial scale due to cultural factors and lower consumption of fish per capita. In Brazil the lack of official regulations to establish standards of identity and quality of the product for commercialization, nationally and internationally, is a limiting factor for commercial scale commercialization (Santo, 2003; Viegas and Guimarães, 2011).

2 Fermentation

The process consists of the osmotic dehydration and maturation of the meat by the action of salt and endogenous enzymes of the fish tissue produced by microorganisms for protein hydrolysis (Essuman, 1992). Anaerobic fermentation slows oxidative biochemical processes and microbial deterioration by preserving food and extending its shelf life (Ogawa and Maia, 1999; Viegas and Guimarães, 2011).

Fermented fish products are developed by salting with varying concentrations of salt with subsequent fermentation. The chemicals formed during the maturation period will determine nutritional and sensorial characteristics of the final product, whose organoleptic properties, especially flavor, aroma, color, and texture will vary according to the method applied, generally geared to the culture and customs of each region, climate, availability of fish or other species of fish, and use and concentration of salt. Salting helps in the selection process of beneficial bacteria, especially lactic acid fermenters (LAB), avoiding the decomposition of the raw material and contamination by pathogenic bacteria (Viegas and Guimarães, 2011).

Currently, varieties of fermented fish products are part of the human diet at the global level. The products can be developed from different species of marine or sweet fish, as well as crustaceans and mollusks, with the use of various ingredients (vegetables, cereals, condiments, spices, wines, pepper) according to the culture of each region, through the application of traditional methods (complete fermentation) or not (incomplete fermentation). Different types of products have improved nutritional and sensory content, and these characteristics are specific to the region of origin (Hall, 2011).

The classification or the way of grouping the different fermented fish products is mainly based on the texture of the final product, divided into three categories according to Viegas and Guimarães (2011):

- Preservation of the natural form of the fish
- Paste obtained by maceration of fish
- Liquid obtained by complete hydrolysis of fish (fish sauce)

3 Types of Commercial Products

In the preparation of fermented fish food, the raw material used in the processing may be whole (containing the head and the viscera), only eviscerated or headless and eviscerated, both forms washed in running water (Ogawa and Maia, 1999). The presence or absence of the viscera and the fish species (fish, crustacean and molluscs) will influence the action of endogenous tissue enzymes in the fermentation process. This also helps in the metabolism of the natural microbiota and in the production of its metabolites present in the final product, resulting in products of different flavor and texture based on the method of production and maturation time (Oetterer et al., 2012). Following are some commercial products of fermented fish and their main places of origin.

3.1 Fish and Crustaceans' Sauce and Paste

Sauce and fish paste are products made in Southeast Asia, Northeast India, Africa (North and West), and Europe. They are used as condiments in the preparation of other foods to confer flavor and are accepted in the commercial market (Hall, 2011).

In Southeast Asia, commercial products are obtained by traditional methods whose fermentation is complete. Such products are obtained on a large scale to meet the demand and supply of a food that contains excellent nutritional value due to the bioavailability of the protein and a viable production cost due to the use of low investment technology. Of particular note are the 'bangoog' and 'prahoc' fermented fish pastes from Philippines, Cambodia, and Indochina. The 'nuoc-mam', 'nam-pla', 'pla-ra', 'pla-som', 'prahoc', and 'pla-jao' sauces are produced in Philippines, Vietnam, Cambodia, Indonesia, and Thailand (Ogawa and Maia, 1999; Hall, 2011; Viegas and Guimarães, 2011). In Figures 14.1, 14.2(a), and 14.2(b), the operational flowcharts for obtaining 'nam-pla', 'plar-ra', and 'pla-som' fermented sauces, respectively, are presented.

The shrimp and crab paste are a product enjoyed in the cuisine of Southeast Asian countries. The processing is similar to that obtained for 'nuoc-man' fermented sauces. The shrimp is

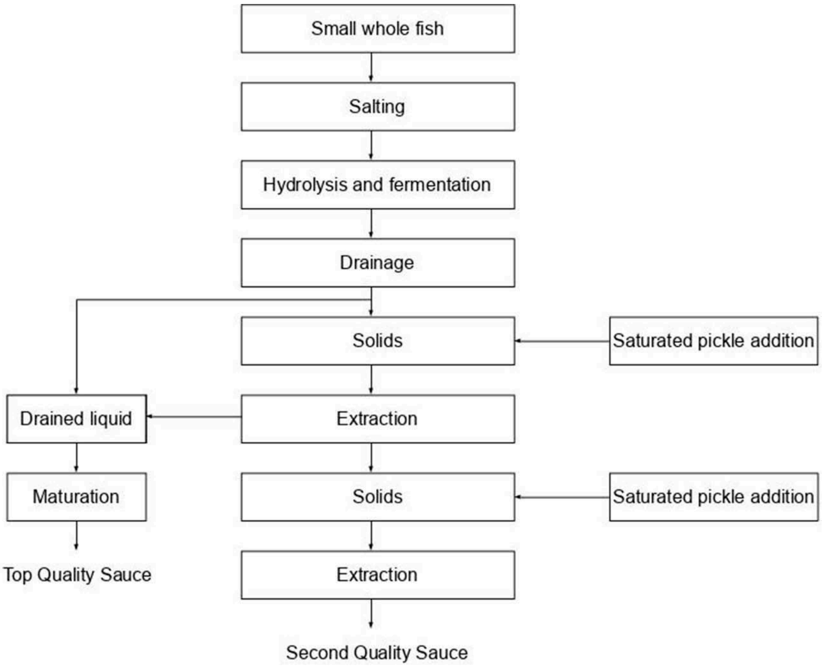

FIGURE 14.1

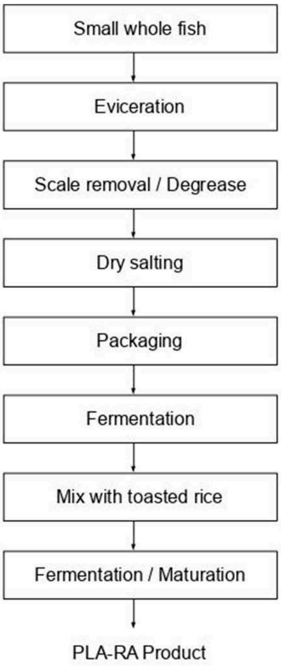

FIGURE 14.2(A)

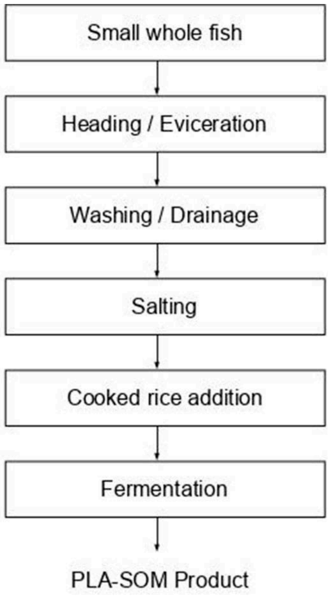

FIGURE 14.2(B)

kneaded and mixed to the salt the concentration of which varies according to the place of production (1 kg salt: 3.0 to 3.5 kg shrimp). They are then filled in pots/clay pots up to the brim to remove air, to avoid decomposition of the product, and then exposed to the sun for 1 to 4 days for drying. The pots are sealed and placed on the floor. The maturation occurs over a period of 12 to 18 months. At the end of the process, the jars are opened and the product obtained is a paste of solid consistency with a pungent odor and taste (Hall, 2002, 2011).

The crustacean paste is used as a seasoning or salt substitute, and according to the place of origin will be give varying names. It is called ‘patis’ in the Philippines, ‘trassi’ or ‘terasi’ in Indonesia, ‘mampla’, ‘kappe’, or ‘kapi’ in Thailand, ‘budu’ or ‘Belachan’ in Malaysia, ‘Ngapi’ in Burma, and ‘mômruôc’ in Vietnam. It is also used in southern China and Bangladesh (Ogawa and Maia, 1999; Berkel et al., 2005). Different products such as sauces and pastas prepared with varying amounts of salt and carbohydrate-rich sources such as rice and soy are generally used as supplements in diets based on soft-tasting cereals (Hall, 2002; Viegas and Guimarães, 2011).

The ‘Bangoog’ pasta is produced with different species of fish such as sardines, mackerel, shrimp, and oysters. The product has a pasty texture and can also be matured before consumption and added with ‘angkak’ (fermented rice product with yeast), which gives the product a typical color. From the liquid produced during the fermentation of the bangoog product, a so-called ‘patis’ sauce is produce (Hall, 2011; Viegas and Guimarães, 2011).

In Northwest India, the ‘ngari’ and ‘hentak’ are fermented fish pastes used in Indian cooking. The ‘ngari’ is produced from the species of fish *Puntius sophore* (called Phoubu) and the ‘hentak’ produced from the species *Esomus danricus.* The largest production is in Manipur; however, fermented fish and crustaceans are widely processed and consumed in the seven states of Northwest India (Singh et al., 2012). According to the locality in India ‘ngari’ can be termed as ‘seedal’, ‘sepaa’, ‘hidal’, and ‘Shidal’ in Assam, ‘Tripura’, ‘Mizoram’, ‘Arunachal Pradesh’, and ‘Nagaland’, while in Manipur, it is called ‘Ngari’. Like ‘ngari’ and ‘hentak’ other fermented foods, such as ‘hawaijar’, ‘soibum’, ‘soidon’, ‘ziang sang’, and ‘Atingba’, are also produced and consumed by local people. In Manipur, ‘ngari’ is the most consumed fermented food and is well accepted by regular consumers, despite its strong and repulsive odor (Singh et al., 2012).

The traditional method is based on simple, handmade techniques inherited from indigenous generations for family consumption. For commercial-scale production, fish is obtained in the dehydrated form of other Northeastern states of India, and processing is based on the traditional method using techniques that allow controlled fermentation. In the 'ngari' processing fish are caught in lakes and rivers and later washed, eviscerated, and dehydrated in the sun for a few days. After drying, they are immersed in water for 10 to 15 minutes to impart soft texture, soaked in vegetable oil, and again dehydrated in the sun for 1 hour. They are then placed into 'clay' pots covering the entire surface of the pot (air removal) and pressed to give the desired texture. The pots are sealed (wet sludge) and buried in the ground or in wells (1/3—one-third of the pot securely attached to the floor) to avoid the effects of high ambient temperature and consequent decomposition of the fish. Fermentation occurs for an extended period, from 3 to 6 months in commercial processing and from 6 to 12 months in the artisanal processing. After the pots are opened, the liquid ('Phumai') present on the surface of the pot is decanted and the 'ngari' is ready to be consumed in the diet presenting a shelf life of 12 to 18 months (Jeyaram et al., 2009; Singh et al., 2012; Keishing and Banu, 2015). The flow diagram of the processing of the 'ngari' fermented sauce is shown in Figure 14.3.

The processing of the 'hentak' is similar to the preparation of the 'ngari' in pre-processing for maturation. The dehydrated fish in the sun is crushed and mixed with pieces of the stem (petiole)

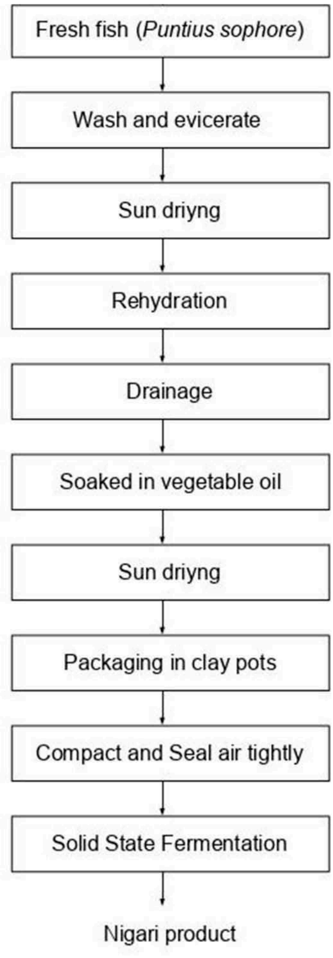

FIGURE 14.3

of *Alocasia macrorhiza* (washed and exposed to the sun for 1 hour) to form a paste. Small portions of the paste are placed in clay pots, air removed by compacting the mixture in the pot, buried in the ground or in wells, and stored for maturation that will occur within 2 weeks. At the end of maturation, the product will present a consistent and firm texture and may be stored for a few months (Jeyaram et al., 2009). The flowchart for the processing of the 'hentak' fermented sauce is show in Figure 14.4.

In Africa, fish fermentation presents different methods from one region to another, where the different concentrations of salt employed and the time of maturation will influence the taste, texture, and appearance of the fermented product (Hall, 2011). In Ghana 'mamone' or 'bomone' are produced with various fish, such as mackerel (*Thyrsitops lepidopodea*), bicuda (*Sphyraena barracuda*), or whiting (*Mycteroperca* spp.), where salted fish (1:9 ratio) is matured from 1 to 7 days and subsequently dehydrated for up to 72 hours. The fermentation period is short and the process occurs incompletely, where the fish muscle is not fully hydrolyzed and the final product will either have its original shape or be cut into pieces, with a mild taste, strong and characteristic odor, used as a condiment in the preparation of soups and cooked food or consumed directly. Some authors have described the product as unpleasant mainly by the non-regular consumer (Hall, 2011). In Gambia and Senegal the 'gaedja' is produced, in Egypt (North Africa) the 'fasikh' is produced, which initially consists of the maturation of the fish eviscerated by endogenous tissue enzymes, followed by dehydration in the sun until the consistency desired for consumption is obtained. Both products are used as condiments in the preparation of other foods. The products have high salt content and strong odor (Essuman, 1992; Hall, 2011). In Sierra Leone, the fish are fermented through the same method, where the maturation with the use of salt occurs for 4 days and then it is followed by dehydration in the sun (Viegas and Guimarães, 2011).

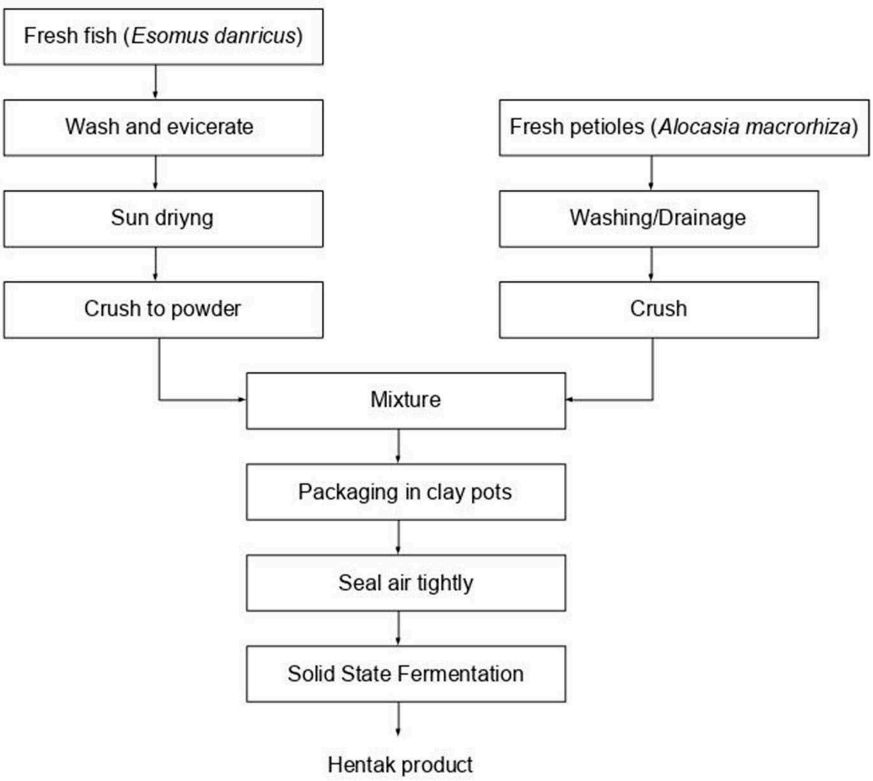

FIGURE 14.4

In general, fish sauces and pastes are the final products of hydrolysis of fish muscle tissue that are prominent in high protein and lipid content. The total amino acid content is approximately the same as that found in fish muscle protein *in natura*, predominantly the amino acids aspartic acid, glutamic acid, serine, alanine, proline, valine, leucine, and arginine. The amino acids aspartic acid and glutamic acid act as flavor enhancers providing umami flavor in various fermented fish products (Hall, 2011; Viegas and Guimarães, 2011).

3.2 Anchovy Fish

In Europe, the method used for the production of fermented fish products is anchovy, and the fish most commonly used for processing on a commercial scale are those made with Engraulidae fish, considered legitimate anchovy products. The species *Engraulis encrasicolus* called European anchovy by FAO is one of the most used in the process. Ogawa and Maia (1999) classify anchovy products as special products made from sardine or herring fish species, those produced in Germany (‘appetistsild’ and ‘krauterhering’), in Scandinavia anchovados prepared with sprat (Sprattussprattus, of the Clupeidae family), and ‘tidbits’ with Norwegian trout cut into pieces; in France it has the ‘patéd’anchois’, the ‘crème d’anchois’, and the ‘beurred’anchois’. In Japan the anchovados (‘Shiokaras’, ‘konovata’, ‘awabi-toshio’) are produced from fish, sea cucumber, squid, and octopus mixed with vegetables such as soybeans and rice are also fermented. In France anchovy products are known as ‘anchovies’, in Spain as ‘anchovies’, in Italy as ‘ancioia’, and in Portugal as ‘anchovies’ (Ogawa and Maia, 1999; Viegas and Guimarães, 2011). In Italy, France, and Portugal anchovy fish products are considered fermented fish and the product name is given from the name of the species used plus the term anchovy or anchovados (Viegas and Guimarães, 2011). In Argentina, Chile, Brazil, and Peru the anchovy products are elaborated from the species of anchovies (*Pomatomus saltator*) and sardines (*Sardinella brasiliensis* = *Sardinella January*) (Santo, 2003; Viegas and

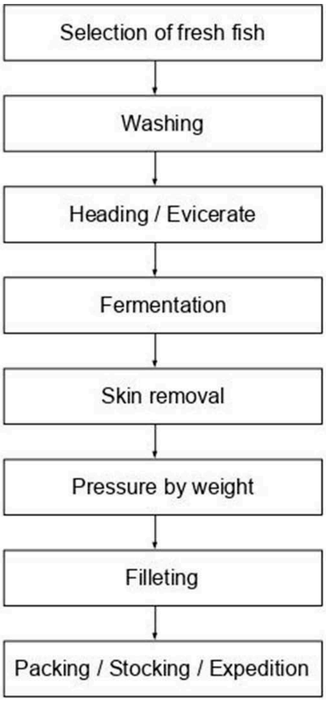

FIGURE 14.5

Guimarães, 2011). The fish used in processing have a high degree of freshness, are washed, headed, and eviscerated, followed by salting in high concentrations (30%) and subsequent maturation for a prolonged period of 4 months. After fermentation, the skin is removed, pressed, filleted, and conditioned in glass containers with edible vegetable oil (Figure 14.5). The product anchovated after fermentation presents desirable sensory attributes, mainly odor and rosy color (Santo, 2003; Viegas and Guimarães, 2011). In Brazil and Portugal, the anchovy product is considered a semiconservative, and the presentation form for commercialization is given according to the common name of the processed species (Santo, 2003; Viegas and Guimarães, 2011).

3.3 Salted Fish and Salted and Dried Fish

Salting consists of one of the oldest preservation methods and is currently used for obtaining a product with typical sensorial characteristics of flavor and aroma to meet the requirements of the international market (Nunes and Pedro, 2011). The basic principle is the osmotic dehydration of its tissues that become permeable to sodium chloride, salt (NaCl), by diffusion, occurring the release of water free of the tissues by osmosis (Ogawa and Maia, 1999).

The traditional method of salting and drying the fish was modified to optimize process time due to shorter maturation or cure time, and the lower salt concentrations used gave the product a softer aroma and palate, a more succulent texture, a sensorial quality differentiated and very well accepted in Europe (Oetterer et al., 2012).

The Nordic countries are the largest producers and exporters of salted and salted and dried products obtained from the processing of fish species belonging to the Family of Gadids (FAO, 2018). Norway, Iceland, and the Faroe Islands are the largest exporters and the main importers are the countries of Southern Europe and Latin America, mainly Portugal and Brazil (Pórarinsdóttir et al., 2010; FAO, 2018).

Cod (*Gadus morhua* from the Atlantic, *Gadus macrocephalus* from the Pacific and *Gadus ogac* from the Greenland) is the main processed and commercialized product being exported to more than 200 countries (Oetterer et al., 2012). Sensory quality varies according to the characteristics of the fish used in the processing, as well as the method of production practiced in the producing countries, involving the techniques of capture and any manipulation given during the capture and post-capture process. Raw material benefited and frozen on board the vessel guaranteeing the high degree of freshness of the fish for processing and a final product with nutritional, sanitary and sensorially accepted quality (Pórarinsdóttir et al., 2010).

The sensorial and commercial quality of the product have undergone recent changes to meet the demands of the international market, especially the younger consumers. Salting procedures and muscle healing conditions modified the organoleptic characteristics (flavor, aroma, texture, and color) of the products and increased the yield by weight of the final product, contributing to improvements in commercial quality (Lindkvist et al., 2008; Nunes and Pedro, 2011). The cure time was reduced resulting in a milder, less accurate flavor, and the whiter looking muscle (Lindkvist et al., 2008; Pórarinsdóttir et al., 2010). However, in some countries, as in Brazil, traditional products containing more salt have a greater acceptance of the market. In Angola and Portugal, consumers prefer cod with an intermediate level of salt, and in Spain and England, the product needs to be less salty. In the Republic of Congo and Angola, the driest products are the most accepted by the regular consumer (Lindkvist et al., 2008; Nunes and Pedro, 2011).

The elaboration, distribution, and consumption of less salty or ‘light’ and cured products have increased in the last decade. Differentiating from traditional products, the saltier ones, by the sensorial attributes conferred (odor and taste) and commercialized price (Lindkvist et al., 2008; Pórarinsdóttir et al., 2010).

‘Light’ products are only injected and either salted and then stored and packaged at low temperatures until they are marketed. Curing will occur during storage and distribution. In addition, the sensitivity of the protein to the lipid and lipid metabolism of the lipids is not due to the long-term cure (2%) (Pórarinsdóttir et al., 2010).

The classification of cod is focused on commercial parameters that define market preferences, higher yields, and final product prices. This is claffied based on the size of the fish, degree of freshness, techniques of capture, method of obtaining, and whether or not subjected to drying. Generally, products that will be destined for drying cannot receive additives such as sorbic acid, sodium sorbate, potassium sorbate, and phosphates (it slows the effects of oxidation), as it increases the water content by prolonging the drying period (Codex, 1989; Þórarinsdóttir et al., 2010).

Sensory methods, through subjective evaluations conducted by trained evaluators, classify the product for export. The general appearance of the product, color (whiteness, yellow), thickness, presence of cracks, hematomas, and blood spots are parameters observed during the analysis. Different patterns of identity and quality are used in Norway and Iceland to serve the different markets (Lindkvist et al., 2008; Þórarinsdóttir et al., 2010). The procedures used to salt the cod also underwent modifications to meet market requirements. The traditional method of stacking fish alternating with layers of salt, called 'kench' (dry salting), with prolonged cure times, serves specific market niches, such as in Portugal (Þórarinsdóttir et al., 2010). Currently, the procedure is divided into several stages, and consists of these: combinations of injection, salting, pickling performed in closed vats with or without addition of water to form brine (the brine is not exposed to fish), drying, and stacking. The concentration of salt used varies from 12% to 24% according to the producer country and the external market demands. Salting of fish is done in the form of fillets or filleting type butterfly (division) and the salt may be added or not with other ingredients. The salting process is reduced taking about 10 days after pre-drying, then packed in sealed cardboard boxes, and stored under refrigeration (below 8°C). For some segments of the market, fish are dry and cured, especially for Portuguese consumers (Þórarinsdóttir et al., 2010).

The use of low temperatures (8°C) throughout the whole process will retard the growth of halophilic microorganisms, lipid oxidation, and will maintain the yield by weight (Ogawa and Maia, 1999). The weight changes during storage are related to the water activity (Aw) in salted fish and the relative humidity (RH) of the air where the product is stored. When RH is greater than Aw (0.75), the salty muscle absorbs water and gains weight. Thus, the equilibrium between the RH and Aw must maintain the quality of the product in relation to the yield by weight (Ogawa and Maia, 1999; Þórarinsdóttir et al., 2010).

4 Nutritional and Sensorial Characteristics of Fish Fermented Products

The evaluation of fermented fish products are based on desirable sensory quality (taste, odor, color, texture, and overall appearance). The taste, odor, and color are the characteristics that stand out in the acceptability of the product fermented by the consumers, be the sauces, pastas, fish anchovados, or salted and salted and dried. The mild or more accurate taste and color are significant in relation to the acceptance of the fermented product. The sauces usually have a light brown coloration, undesirable and attractive by the consumer, being an important parameter of quality. In relation to cod, the white and moist (succulent) muscle has been the major trend in the international market (Þórarinsdóttir et al., 2010).

The taste and color of the product depend on the species of fish used in the processing classified as lean muscle such as lean (up to 2% total lipid) or fat (above 2% total lipid), as well as the method (Ogawa and Maia, 1999). The texture of the fermented or cured product varying with the type of fermentation (complete or incomplete), species of fish (lean or fat), curing time, and should be smooth and soft but consistent for whole and salty fermented fish and dry, and for the slightly salty soft and juicy (Þórarinsdóttir et al., 2010; Viegas and Guimarães, 2011). Such characteristics are obtained when the products are processed from fatty species. Fish species are considered to be lean generally when used in the manufacture of fermented products or they provide a firm and dry texture and less accurate taste, with less acceptance by consumers (Ogawa and Maia, 1999).

In relation to the nutritional value, the highlight is for the content of proteins and free amino acids present in the fermented product of fish, especially in the product sauce and fish paste. They are rich in amino acids (Glycine, Alanine, Lysine, Leucine, and Valine and source of soluble proteins, 50% to 60%) that are bioavailable and formed by protein hydrolysis controlled by the action of endogenous enzymes and or produced by beneficial microorganisms (Oetterer, 1999). They are also rich in organic acids, such as acetic, lactic, and succinic, which confer a slight acidity (pH 5, 6.3) and stability to the final product. Sensorially the presence of free amino acids confers the flavor and stimulates the taste receptors like glycine, alanine, and glutamate, evidencing the nutritional and sensorial quality of the fermented products for a nutritious and balanced diet (Hall, 2011).

In salted or salted and dried products, the use of salt and the temperatures used for drying modify the structure of the lipid favoring the oxidative process. The higher the lipid content in fish muscle the greater the degree of oxidation (Nunes and Pedro, 2011; Pedro and Nunes, 2011). Some particularities related to fish species, time of year, physiological state, and feeding will interfere in the chemical content of the muscle and consequently in the processing, where it is necessary to estimate a shorter shelf life during storage in order not to compromise product quality and damage to health. Alterations caused by rancidity alter the color (rancid, yellow, or orange) and off-product during storage (Ogawa and Maia, 1999; Menegassi, 2011).

5 Sanitary Safety of Fermented Fish Products

The fermentation process of fish creates inhibitory conditions for most foodborne pathogens. However, if industry and artisanal producers neglect hygienic-sanitary control of the process, problems related to deteriorating microorganisms and the production of biogenic amines, especially histamine, may occur. In this sense, official programs and controls are in place to ensure product safety.

One of these controls is the adoption of the Hazard Analysis and Critical Control Points (HACCP) system, whose development, implementation, and execution are the responsibility of the company that manufactures the product. This system has as its guideline the preventive character, based on a risk analysis, which identifies potential hazards and preventive measures to eliminate, reduce, or prevent the occurrence of the hazard. A guide on fish risk analysis and the development of an HACCP plan is addressed in the FDA Guidelines (2001) and Ryder et al. (2014). The European Union, through Resolution (EC) No. 854 of April 29, 2004, established the official control of animal products for the Hazard Analysis and Critical Control Points (HACCP) system audit. In Brazil, this system was established through Administrative Rule no. 46 of 1998 (Brasil, 1998).

The verification of freshness attributes should be performed on fresh, cooled, and frozen products (Brasil, 2017a). In addition, complementary tests such as determination of histamine (in fish susceptible to histamine production), pH, total volatile bases (BVT), levels of trimethylamide (TMA), and trimethylamide (OTMA) should be performed to ascertain the quality of the material fermentation process (Tavares and Gonçalves, 2011). The formation of BVT, TMA, and OTMA are results of the fish deterioration process. Essuman (1992) describes that this process is dynamic and sequential, involving five chemical changes.

- Enzymatic degradation of nucleotides and nucleosides in the meat, leading to the formation of inosine and hypoxanthine, responsible for the tasteless and bitter taste, respectively.
- Bacterial reduction of OTMA, a non-volatile and odorless compound, to TMA, which has an ammoniacal odor.
- Dimethylamine Formation (DMA).
- Protein breakdown with subsequent formation of ammonia (NH_3), indole, and hydrogen sulphide.
- Oxidative fat range.

Therefore, the hygiene of the process, the equipment, and the manipulators must be constantly monitored, together with the official controls previously mentioned, so that the raw material destined to obtain the fermented products of fish is safe and, thus, preserving the health of public.

6 Microbiota of Fermented Fish

The natural microbiota of the fish is composed of quantitative and qualitative bacteria, and the rules of hygiene are fundamental so that the addition of other bacteria doesn't occur. Thus, fish from tropical waters tend to have a richer microbiota in Gram-positive mesophilic bacteria. In contrast, those with cold water harbor more Gram-negative bacteria (Jay, 2005). In this sense, Vieira (2011) states that regardless of whether the fish is of marine or freshwater origin, it will reflect the place from where it was captured, and that population of microorganisms will be proportional to the pollution of their habitat.

The fermentation process of the fish modifies the intrinsic characteristics of the product, such as pH and water activity (Aw). The fall of these parameters ends up acting as a selective factor for certain bacteria. An example is lactic acid bacteria (LAB), which are predominant in fermented fish products (Paludan-Müller et al., 2002), whose role is to ferment the available carbohydrates and thus promote a decrease in pH (El Sheikha et al., 2014).

In relation to the pathogens, the high concentration of salt in the fermented products hinders its growth. However, the halophilic group, microorganisms capable of growing in high amounts of salt, are favored, for example *Pediococcus halophilus*, which has the ability to produce histamine during long storage periods at temperatures from 20°C to 25°C (Jay, 2005). Gassem (2017) evaluating the microflora associated with fermented salted fish from the Jazan region of Saudi Arabia found a variety of species such as aerobic, halophilic, *Staphylococcus* bacteria and molds and yeasts. However, pathogenic species, such as *Vibrio* sp., *Listeria monocytogenes*, *Yersinia* sp., and *Campylobacter* and halophilic bacteria, such as *Halobacterium* sp. and *Halococcus* sp., are responsible for the production of red pigments in salty products.

Anihouvi et al. (2007) found a wide variety of gram-positive and gram-negative microorganisms associated with the fermentation of Lanhouim (a product of African origin) such as *Bacillus* sp., *Staphylococcus* species, mainly *S. lentus* and *S. xylosus.*

Reduction of pH in fermented fish is an important limiting factor for the growth of some pathogens such as *Clostridium botulinum* type A and B, *Listeria monocytogenes*, and *Vibrio* sp. All types of *C. botulinum* are inhibited with concentrations between 10% and 12% of salt and at pH of less than 4.5. In addition, botulinum toxin is inactivate by enzymatic proteolysis in the fish fermentation process (Essuman, 1992). However, it is important to note that not all fermented fish products have low pH values and high salt concentration, which is essential to control the process variables in order to avoid contamination by pathogenic microorganisms.

Therefore, the hygiene practices in fish handling and the temperature controls in the various phases of the process become essential for the microbiological quality of the product.

7 Toxicology and the Production of Histamine

The production of histamine, a biogenic amine, in fish occurs due to the presence of the enzyme histidine decarboxylase, which decarboxylates the amino acid histidine, present in free form, forming histamine (FDA, 2001; Gonçalves, 2011). The enzyme is produced by some species of microorganisms such as *Enterobacteria*, *Clostridium*, *Lactobacillus*, *Morganela morgani*, *Klebsiella pneumoniae*, *Proteus vulgarius*, and *Hafnia alvei* (Frank, 1985; Lehane and Olley, 2000).

Scombrotoxicosis, as histamine intoxication is known, occurs more frequently in species of the *Scombrideae* family, such as tuna, mackerel, and bonito. However, sardines and anchovies have also been reported in cases of deterioration of fish by histamine (Essuman, 1992;

Gonçalves, 2011). Since the formation of biogenic amines, such as histamine, occurs through bacterial enzymes, hygiene in obtaining raw material, evisceration on board, rapid cooling of fish become essential measures to avoid contamination by producing bacteria of histamine (Gonçalves, 2011).

Formation is faster at higher than cooling temperatures. Therefore, high temperature is more commonly associated with histamine generation than long storage, which is usually so at lower temperatures and is linked to changes in organoleptic characteristics (FDA, 2001; Gonçalves, 2011). In addition, the production of biogenic amines is primarily related to marine fish and species of the scombrideae family and the use of freshwater species as a raw material is not a potential hazard (Huss et al., 2003).

The legislation of different countries establishes parameters for the maximum amount of histamine allowed in fish. The European Union, by Regulation (EU) Nª 1019/2013 of 23 October 2013, fixed the maximum limit of 200 mg/kg for fishery products of fish species with a high histidine content, and of 400 mg/kg for fishery products subject to enzymatic maturation in brine made from fish species with a high histidine content; and 400 mg/kg for fish sauce produced by fermentation.

In Brazil, the Ministry of Agriculture, Livestock and Supply (MAPA) establishes the maximum limit of 100 mg/kg based on a sample composed of nine sampling units for families of histamine-forming fish. However, no sample unit may exceed the amount of 200 mg/kg histamine (Brasil, 2017b).

The fermentation process of the fish can facilitate the conditions for the formation of histamine, due to the following factors: free amino acid availability, presence of decarboxylase positive microorganisms, conditions for microbial growth, synthesis, and decarboxylase activity (Ostergaard et al., 1998).

In addition to the control of hygienic sanitary conditions during manufacturing, histamine control has been proposed by different methods such as high hydrostatic pressure, irradiation, use of additives, and modified atmosphere. However, in recent years the use of microorganisms that have enzymes that degrade histamine has become an emerging method to reduce the concentration of this amine, especially in fermented fish products (Mah and Hwang, 2009; Naila et al., 2010).

Huang et al. (2015) studied the action of *Bacillus polymyxa* D05-1, isolated from salted fish, on the degradation of histamine and concluded that the bacterium degraded 100% of the histamine present in TSB broth, being considered a halotolerant bacterium that degrades histamine and produces histamine dehydrogenase to break it down. Kongkiattikajorn (2015) reports that the use of stater cultures in a fish fermentation reduced the accumulation of biogenic amines, including histamine, concluding that a mix of cultures of *Lactobacillus sakei* KM5474 and *Lactobacillus plantarum* KM1450 increased the effect of reducing the amount of biogenic amines.

Sukarno et al. (2017) tested fungal species associated with turmeric (*Curcuma longa* L) to inhibit histamine-forming bacteria in fish and concluded that several species of fungi isolated from turmeric had the potential to inhibit the growth of *Morganella morganii*, an important histamine-forming microorganism.

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Section 5

Milk-Based Fermented Food Products

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Kephir (Kefir): Fermented Dairy Product

Didem Deliorman Orhan and Sultan Pekacar
Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey

1 Introduction

Today, because of the increasing educational levels of consumers and their changing living standards, demand for functional foods is increasing day by day (Erbaş 2006). Foods consumed in the pharmaceutical formulations in daily diet, without synthetic content, with high nutritional value, reduced risk of disease, promoting physiological health and well-being in humans are defined as functional food (Erbaş 2006). Probiotics and prebiotics are also classified as functional food. Probiotics are defined as the living organisms that regulate the intestinal microbial balance. Prebiotics are known as the nondigestible and fermentable carbohydrates that facilitate the colonization of nonenteropathogenic bacteria, which increase the activity of colon bacteria. In addition, prebiotics are also considered as the nutritional sources of probiotics (Inanç, Şahin, and Çiçek 2005). Probiotics are most commonly consumed as fermented dairy products. Fermented foods have been the main products in nutrition since the beginning of the history of humankind (Marco et al. 2017). In recent years, scientific studies on the production, consumption, and nutritional and health benefits of kefir with probiotic properties have increased (Karabıyıklı 2016). In a recent report, positive contribution to human health is shown as one of the most important factors for the increase in demand for fermented foods (Marco et al. 2017). According to the Turkish Food Codex Communique on Fermented Milk Products, kefir is defined as a fermented milk product that uses starter cultures or kefir grains containing specific strains of *Lactobacillus, Leuconostoc, Lactococcus.* and *Acetobacter*; lactose fermenting yeasts (*Kluyveromyces marxianus*); and nonlactose fermenting yeasts (*Saccharomyces unisporus, Saccharomyces cerevisiae*, and *Saccharomyces exiguus*) for fermentation (Anonim 2009).

Kefir, the traditional fermented milk product, is thought to be consumed primarily in the Caucasus Mountains, Tibet, and Mongolia. In the North Caucasus, kefir is produced by the local people by using cow and goat's milk and is very refreshing. The importance of kefir was understood as a result of research by Russian scientists on probiotic bacteria in the 1920s. The researchers found that there were two probiotic bacteria in yogurt but this number increased to 25–30 in kefir. In the Caucasus, it is stated that due to its nutritional value and therapeutic properties, kefir is produced in leather overalls and oak barrels, and is being produced in Eastern and Central European countries since the late 19th century (Karatepe and Yalçın 2014).

It is believed that the word kefir is derived from the word 'Keyf', which means delightful and enchanting in Turkish (Tomar, Çağlar, and Akarca 2017). Kefir is also known as kefyr, kephir, kefer, kiaphur, knapon, kepi, and kippi (Sarkar 2007). It is a fermented dairy product prepared by fermentation of ethyl alcohol and lactic acid by adding kefir grains to the milk. It contains carbon dioxide and has a slightly acidic and mild alcoholic refreshing taste, and is different from other fermented dairy products because it is obtained as a result of both lactic acid and ethyl alcohol fermentations. Kefir, which is formed as a result of metabolic activities of different kinds of bacteria and yeasts in grains, has a special flavor and aroma. Lactic acid, CO_2, ethanol, and other products obtained during the fermentation process are effective in the development of the taste and aroma of kefir. In fermentation, yeast, lactic acid, and some acetic acid bacteria cause a series of biochemical changes. As a result of this process, kefir becomes sour, slightly alcoholic, viscous, and frothy (Tomar, Çağlar, and Akarca 2017).

2 Kefir Grains

Kefir is a beverage produced by fermentative activation of kefir grains containing different amounts of yeast, lactic acid bacteria (LAB), and semi-solid granulated acetic acid bacteria having a polysaccharide matrix (Guzel-Seydim et al. 2011). These kefir grains are small particles with yellowish white color, irregularly shaped, at least 3 to 20 mm in diameter are reminiscent of cauliflower or popcorn (Tomar, Çağlar, and Akarca 2017).

After the kefir grains are incubated in milk (at 25°C) for about 22 hours, the microorganisms in the grains continue to grow together with the formed lactic acid and other flavor components as a result of physicochemical changes in milk. The resultant product, kefir, is a fermented dairy beverage with a refreshing and distinctive taste, containing lactic acid, carbonate, carbon dioxide, acetaldehyde, acetone, and a small amount of alcohol. The most important feature that differentiates kefir from other fermented foods is that kefir grains can be reused after fermentation (Guzel-Seydim et al. 2011).

Microflora of kefir grains contains LAB, *Acetic acid* bacteria, and yeasts. Some yeasts found in kefir can ferment lactose, some of them cannot ferment lactose. Microorganisms found in kefir are found in its different parts. At the bottom of the kefir, there are yeasts that cannot ferment lactose, and those which ferment lactose are in the middle layer. The surface of the kefir has mesophilic LAB, *Streptococci,* mesophilic and thermophilic *Lactobacilli*, and *Acetic acid* bacteria (Tomar, Çağlar, and Akarca 2017).

The diversity of the population in the microflora of kefir grains, the type and the number of microorganisms, the biochemical properties and the microbiological profile of the milk used, or the methodological differences used in production, lead to changes in color, odor, and taste. These parameters are also effective in the chemical or microbiological composition of the final product (Karabıyıklı 2016).

The kefir grains include a polysaccharide produced by *Lactobacillus kefiranofaciens*, called kefiran, which has a heteropolysaccharide structure containing equal amounts of glucose and galactose. This polysaccharide has a function that can strengthen the viscosity and viscoelastic properties of milk. Kefiran also increases the apparent viscosity of the finished product (Prado et al. 2015; Tomar, Çağlar, and Akarca 2017).

The polysaccharide named kefiran has higher antitumor, antifungal, antibacterial, antiinflammatory, immunomodulatory or epithelial protective, wound healing, and antioxidant effects as compared to other polysaccharides. Today, polysaccharides are important for the food, pharmaceutical, and cosmetic industries. On the other hand, it is a known fact that they are also used as bio-absorbers, metal removers, and bioflocculants.

Many microorganisms, such as bacteria and fungi, are capable of producing polysaccharides. These polysaccharides may be soluble or insoluble. Exopolysaccharides (EPSs) produced by LAB are included in the composition of foods because of their suitable physicochemical characteristics. Kefiran is an EPS classified as a heteropolysaccharide with high glucose and galactose content. This EPS with a water-soluble glucogalactan structure is suitable to be used as a food additive (Prado et al. 2015).

3 Kefir Production

There are various methods used in the production of kefir. In kefir production, cow, goat, or sheep's milk, coconut, rice, or soy milk can be used. There are a variety of options for milk, such as pasteurized, nonpasteurized, full-fat, low-fat, and skimmed (Otles and Cagindi 2003).

3.1 Traditional Process

Kefir production by traditional method begins with the addition of kefir grains to the milk directly.

Raw milk is boiled and cooled to 20–25°C, and then it is inoculated with 2–10% of kefir grains. After fermentation at 20–25°C for 18–24 hours, the kefir grains are filtered off with the help of a sieve and can be dried at room temperature, and kept cold for subsequent use. Kefir is stored at 4°C (Otles and Cagindi 2003).

3.2 Industrial Process

In industrial process, different methods can be used for kefir preparation, but the principles of all the methods are the same. First of all, the dry matter content of the milk is homogenized to 8% and then heat-treated at 90–95°C for 5–10 minutes. It is then cooled to 18–24°C and inoculated with 2–8% kefir culture (bacterial starter) in tanks. The fermentation period varies from 18 to 24 hours. The clot is separated by the pump and distributed to the bottles. Kefir is left to mature at 12–14°C or 3–10°C for 24 hours and then stored at 4°C (Otles and Cagindi 2003).

The main products of kefir fermentation are lactic acid, ethanol, and CO_2. Minor components such as acetaldehyde and amino acid contribute to the aroma of kefir. During fermentation, bacteria and yeasts living symbiotically promote each other's functions. LAB tend to grow faster than yeasts due to their ability to ferment lactose in milk. Thus, these bacteria provide a suitable environment for the growth of yeast. On the other hand, yeasts synthesize complex B vitamins by hydrolyzing milk proteins in order to produce CO_2 and ethanol using oxygen. Bacteria provide the energy necessary for the yeast, while yeasts contribute to the formation of vitamins and amino acids, which are necessary for bacterial growth and multiplication (Tomar, Çağlar, and Akarca 2017).

In the presence of *Propionibacterium* species, there is a significant increase in the amount of vitamin B12. During fermentation, the amounts of vitamins B1, B2, and folic acid are slightly increased (Karatepe and Yalçın 2014).

The researches show that the microbial flora of the kefir grain remains alive in the final product, since no thermal treatment was given to it post fermentation. The number of LAB that make up most of the microbial flora of the kefir tends to increase during the fermentation period. The increase in the amount of bacteria is slow in the early stages of fermentation, and the process also shows a tendency of slowing down toward the end of the fermentation (Tomar, Çağlar, and Akarca 2017)

The yeast activities during the maturation stage of the kefir cannot be stopped like the bacterial activities. This leads to an increase in the amount of carbon dioxide due to the yeast activities during the storage of kefir. Since excessive production of carbon dioxide may cause undesirable taste and aroma in kefir, the increase in carbon dioxide during fermentation should be checked. For this purpose, the amount of yeast in the starter cultures obtained from kefir grain is limited. Therefore, most of the methods to improve kefir production are related to starter cultures (Tomar, Çağlar, and Akarca 2017).

During the fermentation, LAB in kefir are more effective than yeast and acetic acid bacteria in the formation of taste–aroma substances. The yeasts in kefir fermentation also play an important role in the formation of especially the acidic and mildly sweet taste. The ethanol and carbon dioxide formed during the fermentation process are responsible for the typical kefir flavor (Tomar, Çağlar, and Akarca 2017).

Aroma of the traditionally produced kefir is the result of the activity of many bacteria and yeasts in kefir grains. Differences exist between the composition and sensory profiles of kefirs produced using conventional and modified starter cultures. The modified kefir with little whey is less acidic and has a creamy aroma (Lopitz-Otsoa et al. 2006). In another study, it was determined that the amount of carbonyl compounds responsible for the taste and aroma produced by kefir starter cultures was higher than those produced from kefir grains (Tomar, Çağlar, and Akarca 2017).

In traditional kefir production, kefir grains are used as the main culture. In conclusion, it is revealed that microflora of both kefir grains and kefir produced from these grains is different from each other. This microflora consists of *Lactobacillus* ssp. (65–80%), *Streptococcus* ssp. (20%), and yeasts (5%) (Tomar, Çağlar, and Akarca 2017).

It is very important that the microorganisms to be used in the production of probiotics such as kefir are found in the normal gut microbiota. More importantly, these beneficial microorganisms, after being taken orally, must stay alive in the intestine. Considering all these factors, the number, type, and viability of the microorganisms in all end products obtained using starter culture or kefir grains is extremely important in terms of the bioavailability of the product (Karabıyıklı 2016).

4 Chemical Composition of Kefir

The composition of kefir is so complex and is not well defined (Arslan 2015). Its composition depends on the source of the milk and its fat content. The production technology is the factor effective in the composition of kefir and the composition of kefir grains or cultures. Major products formed during fermentation are lactic acid, acetic acid, pyruvic acid, propionic acid, butyric acid, hippuric acid, CO_2, alcohol, diacetyl, and acetaldehyde. These compounds give taste and aroma of kefir. Diacetyl is produced by bacteria such as *Leuconostoc* spp. and *Streptococcus-lactis* subsp. *diacetylactis*. The content of carbon dioxide in the fermented kefir product increases due to the increase in kefir grain. The desired carbon dioxide concentration in kefir should be 1.98 g/l. The pH value of the kefir is 4.2 to 4.6 (Otles and Cagindi 2003). L(+)-lactic acid derived from lactose in milk is the most abundant organic acid in kefir (Arslan 2015). Kefir consists of 86% moisture followed by 6% sugar, 3.2–3.4% fat, 3% protein, and 0.7% ash. As compared with commercial yogurt, kefir contains twice as much the amount of pyruvic acid, nine times as much acetic acid, half as much orotic acid, and about the same amount of uric acid (Ahmed et al. 2013).

Storage conditions affect the composition of kefir. It has been observed that the concentration of ethanol, acetaldehyde, and aceton could be decreased at a storage temperature of 48°C. However, no decrease in diacetyl levels was observed during storage at the same temperature (Ahmed et al. 2013).

5 Nutritional Character of Kefir

Protein, vitamins and mineral content, and other fermentation products of kefir are related to its nutritive value. (Sarkar 2007). Additionally, it contains high amounts of amino acids, protein, phosphorus, and calcium (Ahmed et al. 2013).

5.1 Vitamin

Kefir contains high doses of vitamins B1 (<10 mg/kg), B2 (<5 mg/kg), and B5 (3 mg/kg), as well as vitamins C, A, and K. The vitamin content of kefir varies depending on the type of milk and microorganism used (Arslan 2015). Some researchers have suggested that while the amounts of vitamins such as pyridoxine, vitamin B12, folic acid, and biotin increase during fermentation, the amounts of thiamine and riboflavin decrease. Other researchers observed that the amount of vitamin B12 in kefir decreases during the process. This reduction was thought to be due to the composition of the specific microflora of kefir grains. The amount of folic acid in the commercially available kefir grains tends to increase after fermentation (Ahmed et al. 2013). Folic acid and pyridoxine content in kefir samples obtained from the milk of different animals was found to be dependent on the amount of fluoride in the water used (Sarkar 2007). It is thought that the decrease of the content of vitamin B12 (34–37%) may be due to the bacteria named *Propionibacterium peterssoni* and *Propionibacterium pituitosum*, which are added to the kefir. On the other hand, while the addition of *Propionibacterium freudenreichii* subsp. *shermanii* resulted in an increase in the content of vitamin B12 (30 times) and folic acid (five times), a slight decrease in the content of pantothenic acid and vitamin B6 was also observed (Sarkar 2007).

Some experiments conducted by the researchers showed that vitamin B12, fat, and protein contents of goat milk kefir fortified with vitamin B12 were stable for 15 days during storage. The pH of this kefir was almost the same as that of the standard kefir (pH 4.2–4.6). However, on the 15th day there was a decrease in the amount of vitamin D3 and in the number of LAB (Dianti et al. 2018).

5.2 Protein

Kefir contains protein compounds that are easily digestible in the body. The amino acid profile of kefir varies during the fermentation of milk, and kefir contains higher amounts of threonine, serine, alanine, lysine, and ammonia as compared to milk. The essential amino acid contents in kefir (mg/100 g) are valine (220), isoleucine (262), methionine (137), lysine (376), threonine (183), phenylalanine (231), and tryptophan (70). Tryptophan, one of the amino acids in kefir, has an important role in the nervous system (Arslan 2015). Whole milk, when used for fermentation, contains relatively less protein as compared to whey and soy milk. Bifidokefir containing *Bifidobacteria* is reported to contain more glutamic acid and threonine when compared to normal kefir (Ahmed et al. 2013).

5.3 Minerals

Considering the mineral content in kefir, it has been found to be a good source of potassium and phosphorus. The macroelement contents present in kefir are potassium, 1.65%; calcium, 0.86%; phosphorus, 1.45%; and magnesium, 0.30%, while the microelements are (mg/kg); copper, 7.32; zinc, 92.7; iron, 20.3; manganese, 13.0; cobalt, 0.16; and molybdenum, 0.33. Researchers found that the ash content of kefir samples ranged from 0.55% to 0.66% (Ahmed et al. 2013).

5.4 Carbohydrates

6% of the kefir's microflora in the gelatinous matrix structure consists of a heteropolysaccharide named kefiran. This polysaccharide that is found as glucogalactan in nature, improves gel formation, rheology, and viscoelastic properties of the gels produced with acidified milk. Gel formation occurs at low temperatures. In addition, kefiran has a biological activity such as *Rotavirus* inhibitory, immunomodulatory, and epithelial cell protective effects (Ahmed et al. 2013). Kefir is a good alternative for people with lactose intolerance who cannot digest a large part of the lactose, which is found as major sugar content in milk. It should also be noted that the lactose content of the kefir decreases while the β-galactosidase enzyme increases at the end of the fermentation (Arslan 2015).

The presence of biogenic amines in the kefir samples was due to the LAB activity. Putrescine, cadaverine, and spermidine were found in all samples. On the other hand, the highest amount of biogenic amine was found to be tyramine (Arslan 2015).

6 Microbiology of Kefir Grains

Kefir grains are obtained from the complex symbiotic relationships among microorganisms such as *Lactobacilli, Acetic acid* bacteria and yeasts. Although the grains used in the production of kefir are formed by lactic acid and alcoholic fermentation, these grains consist of microorganism assemblage, which are held together by the polysaccharide matrix similar to cauliflower (Karatepe et al. 2012).

There are many microorganisms such as yeasts (*Kluyveromyces*, *Candida*, *Torulopsis* and *Sacharomyces* spp.), *Lactobacilli* (*L. brevis*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. delbruecki*), *Streptococci* (*Streptococcus salivarus*), *Lactococci* (*Lc. lactis* ssp. *thermophils*, *Leuconostoc mesenteroides* and *L. cremoris*), and *Acetic acid* bacteria in this symbiosis group. Homofermentative *Lactobacillus* (*Lactobacillus kefir*) species are the most important part of the bacterial flora. In recent years, a new *Lactobacillus* species, *L. kefiranofaciens*, has been identified in kefir. It has been reported that the outer polysaccharide layer of kefir is produced by *L. kefiranofaciens.* This polymer, known as kefiran, consists of live and dead microorganisms with 30–34% casein, 45–60% saccharide, 3–4% fat, and it also contains equal amounts of glucose and galactose; at least 25% of the kefir grains are formed from this polymer.

L. kefir are located in a small area on the surface layer of kefir, while *L. kefironofaciens* are located in the center and are on the entire upper surface of the kefir grains. Apart from *Lactobacillus* species, there are homofermentative and heterofermentative lactic acid, streptococci, acetic acid bacteria, lactose-fermenting, and nonlactose-fermenting yeasts (*Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, *Candida kefir* and so on) in kefir grains (Karatepe et al. 2012).

Generally, 65–80% of the microflora in kefir grains is *Lactobacillus* species (homofermentative, heterofermentative, mesophilic, or thermophilic). 25% of the microflora also consists of *Streptococcus* species and yeasts. LAB produce lactic acid, CO_2 and ethanol. Acetic acid bacteria help to maintain the symbiosis in kefir grains and increase the viscosity of kefir. Yeasts help to maintain symbiosis and help in the formation of CO_2, and develop the characteristic taste and aroma (Karatepe et al. 2012).

The kefir grains used for making kefir have a complex flora. The grains are composed of several microorganisms. These microorganisms are classified under four main groups: homofermentative and heterofermentative LAB and lactose-fermenting and nonfermenting yeasts (Karatepe et al. 2012).

6.1 *Lactic Acid* Bacteria

The LAB are added to the milk as starters to initiate fermentation. These microorganisms produce lactic acid from milk sugar and lower the pH. The LAB, such as *Lactobacillus, Streptococcus, Leuconostoc, Pediococcus,* and *Lactococcus*, are being used as starters for the production of the fermented milk for a long time (Karatepe et al. 2012).

6.1.1 Lactococcus *ssp.*

Lactococcus ssp. are the mesophilic microorganisms used for the production of acids in the fermentation of milk. These microorganisms ferment sugar in a homofermentative manner, proliferate at 10°C and have weak proteolytic activity. *Lactococcus lactis*, which has three subtypes called *Lc. lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis,* and *Lc. lactis* ssp. *hordniae*, is the important species in milk starter cultures. *Lc. lactis* ssp. *cremoris* and *Lc. lactis* ssp. *Lactis* are occasionally isolated from kefir grains (Karatepe et al. 2012).

6.1.2 Leuconostoc *ssp.*

Leuconostoc ssp. are heterofermentative mesophilic coccus found in dairy products, meats, and various fermented foods. This genera as heterofermentative starters has the ability to ferment lactose in milk and produces more D(+) than L(−). *Leuconostoc* ssp. are used to produce diacetyl, CO_2, and acetone from citrate in the dairy industry, and there are seven known species. As the milk starter culture, usually *L. lactis* is used in the fermentation process (Karatepe et al. 2012).

6.1.3 Streptococcus *ssp.*

In total, there are 27 species of *Streptococcus*, but *Strep. thermophilus,* which is a homofermentative bacterium, is the most important species for fermented milk production and produces L(−)lactate (Karatepe et al. 2012).

6.1.4 Enterococcus *ssp.*

Enterococcus ssp. having an ovoid shape are usually of intestinal origin, and rapidly produce acid. Especially *E. faecalis* and *E. faecium* are opportunistic pathogenic microorganisms. *Enterococcus* ssp. are believed to have positive effects on the flavor of kefir. But still there are discussions about them being the starter bacteria (Karatepe et al. 2012).

6.1.5 Lactobacillus *ssp.*

Lactobacillus ssp., the largest group of LAB, are long, thin, curved, short rod shaped, and typically aerotolerant anaerobic, Gram-positive bacteria. Also, these species with the complex nutritional requirements are nonspore-forming bacteria having acidic or acidophilic properties. Some *Lactobacillus* ssp. may contain pseudocatalase enzyme (Karatepe et al. 2012).

Lactobacillus ssp. are homofermentative (produce more than 85% lactic acid) and heterofermentative (produce lactic acid, CO_2, ethanol, and/or acetic acid). There are 64 species of these organisms that produce the two isomers of lactate. Obligate homofermentative LAB (*L. acidophilus, L. helveticus, L. delbruecki* etc.) lead to the fermentation of hexose to lactic acid but cannot ferment pentoses. Facultative heterofermentative LAB (*L. casei, L. plantarum*) can ferment almost all of the hexoses to lactic acid through glycolysis and are also capable of fermenting pentoses to ethanol, lactic acid, formic, and acetic acid through phosphoketolase. Other heterofermentative LAB such as *L. bifermentas, L. fermentum,* and *L. brevis* can ferment hexose to lactate, acetic acid, CO_2, and ethanol (Karatepe et al. 2012).

6.2 Yeasts

Yeasts play an important role in the production of ethanol and CO_2 in fermentation of kefir. Kefir grains generally contain lactose fermenting yeasts (*Kluyveromyces lactis, Kluyveromyces marxianus, Torula kefir*) and also nonlactose fermenting yeasts (*Saccharomyces cerevisiae*) (Karatepe et al. 2012).

Yeasts that are extensively found in nature are unicellular, facultative anaerobic organisms, and have a typical spherical or oval shape. These organisms rapidly convert sugars to CO_2 and H_2O in aerobic condition, while they produce ethanol and CO_2 in anaerobic condition (Karatepe et al. 2012).

Yeasts are widely available in milk and dairy products. These organisms are considered as important contaminants in fermented milk products, such as sour milk and yogurt with acidic pH. *Kluyveromyces marxianus* and *Saccharomyces* sp. are yeast organisms that cause deterioration of fermented products. This is especially important for yogurts with sugar added. However, yeasts in dairy products such as kefir and kımız do not contribute much to the fermentation process. Yeasts have been reported to play an important role in the occurrence of symbiosis between microorganisms in kefir grains and provide significant contribution to the production of CO_2 and its unique flavor and aroma (Karatepe et al. 2012).

7 Health Effects of Kefir

Kefir is an effective functional food for health because it has antibacterial, immunological, antitumoral, and hypocholesterolemic effects. In addition, it has been reported that it is used as a supportive treatment for tuberculosis, cancer, and gastrointestinal disorders in many regions of the world. In the former Soviet Union, it has also been known that kefir was used in the treatment of metabolic disorders, atherosclerosis, and allergic diseases in hospitals and sanatoriums. It is reported by a number of researchers that, on a regular basis, daily consumption of half a liter of kefir has positive effects on the liver, bile, kidney functions, and blood circulation. Also, it is useful in nervous disorders, loss of appetite, and insomnia. Orotic acid content is effective in inhibiting the biosynthesis of cholesterol, in protecting the liver, and in the synthesis of nucleic acids. As a result of kefir consumption, the increase in orotic acid level in the blood is an evidence that kefir is absorbed very well from the intestine (Karatepe and Yalçın 2014).

It has been reported by researchers that kefir and kefir grains have anticarcinogenic and immunostimulant effects. Kefir has antimicrobial activity against Gram-positive and Gram-negative bacteria and some types of fungi. Microorganisms found in the kefir grains prevent the development of pathogenic microorganisms by producing lactic acid, antibiotics, and bacteriocin. In addition, the researchers reported that kefiran (polysaccharide matrix surrounding the granules) has antimicrobial, antimycotic, and antitumoral effects. Karatepe and Yalçın (2014) showed that people (100 people in 25–65 years age range) who consume kefir for 6–9 months have exerted a significant reduction in their gastrointestinal and blood pressure problems, insomnia, and depression symptoms.

7.1 Antimicrobial Effect

Lactic acid, acetic acid, and H_2O_2 formed in kefir exert an antibacterial effect against pathogenic bacteria such as *E. coli* and *Salmonella* spp. It has been pointed out by researchers that lactic acid may create an environment unsuitable for the development of other bacteria by lowering the medium pH. H_2O_2 contributes to the antimicrobial effect of kefir by an antagonistic effect against intestinal pathogens. Acetic acid is also reported to have antibacterial effect. In another study, it was found that kefir grains showed antimicrobial activity against *E. coli*, and this effect was found to be caused by lactic acid and acetic acid released during fermentation (Garrote et al. 2000; Karatepe and Yalçın 2014).

To assess the antimicrobial effect of kefir suspension (0.1 ml) and purified kefiran (50 μg/ml), several bacteria (*Staphylococcus aureus* ATCC 6538*, Streptococcus salivarius* ATCC 39562, *Streptococcus pyogenes* ATCC 17568, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028*, Listeria monocytogenes* ATCC 4957, and *E. coli* ATCC 8739), *Candida albicans*, and agar diffusion method were used (Rodrigues et al. 2005). Both kefir and kefiran are effective against all microorganisms tested but the highest effect was observed against *Streptococcus pyogenes*. The mean values for kefiran and kefir inhibition zones were ranged from 26.3 ± 2.1 to 28.0 ± 2.0 mm, and in the order *S. pyogenes*<*S. aureus*<*S. salivarius*<*S. typhimurium*<*L. monocytogenes*<*P. aeruginosa*<*E. coli*. Ceftriaxone, ampicillin, azithromycin, and oxacillin were used as reference antibiotics.

Kefir exhibits antibacterial activity against Gram-positive cocci, Staphylococcus, and Gram-positive bacilli. Kefir grains display a higher antibacterial activity than the kefir. At the same time kefir has antifungal activity against *Candida*, *Saccharomyces*, *Rhodotorula*, *Torulopsis*, *Microsporum,* and *Trichopyton* species (Karatepe and Yalçın 2014).

7.2 Anticancer Effect

The mechanisms of anticancer effect of kefir are as follows:

1. Reduces mutation and DNA damage.
2. Reduces the activity of a number of enzymes (ß-glucuronidase, nitroreductase, and azoreductase).
3. Inactivates mutagens.
4. Increases the production of short-chain fatty acids and increases acidity,
5. Accelerates apoptosis.

Specific cultures isolated from kefir have been shown to bind mutagenic agents such as indole and imidazole. In a study investigating the anticarcinogenic effect of kefir and kefir grains, it has been reported that microorganisms in kefir reduce the activity of fecal bacterial enzymes and consequently decrease the risk of colon cancer. Selenium, vitamin E, catalase, and superoxide dismutase enzyme content in kefir shows antioxidant effect on cancer cells. This effect is also considered as an anticarcinogenic factor (Karatepe and Yalçın 2014).

In a previous study, from a water-soluble polysaccharide isolated from the kefir grains, KGF-C, was purified and administered orally (0.02–0.1% with drinking water) or intraperitoneally at a dose of 0.05–2 mg per day to the mice. KGF-C has been reported to inhibit the growth of Ehrlich carcinoma cells by 40–64% and Sarcoma 180 cancer cells by 20–90% (Karatepe and Yalçın 2014).

In another study on antitumoral effect of kefir, fusiform cancer cell-transplanted mice were given 0. 5 ml of kefir per day for 20 days, intraperitoneally. Consequently, significant reduction in tumor size was observed. It was also found that kefir was also effective in removing tumor-induced necrosis. No tumor cells were observed in two of the mice who were given 0.5 ml of kefir for 20 days, while the tumor size was reduced in five mice, and no change in tumor size was observed in four mice. The mean tumor size before treatment was 0.06 ± 0.05 cm^3 and the mean values after treatment were found to be 0.02 ± 0.02 cm^3. Reduction in tumor size was found to be statistically significant ($p<0.05$) by researchers (Cevikbas et al. 1994; Karatepe and Yalçın 2014).

7.3 Effects on the Immune System

The effect of kefir on the immune system is thought to be mediated via sphingomyelin isolated from the lipids found in kefir. This effect has been demonstrated by *in vitro* and *in vivo* methods. It has been found that LAB present in kefir increase nonspecific resistance to tumors or infections in humans or animals, and additionally exert a potent effect on specific immune reactions (Karatepe and Yalçın 2014).

LAB have an adjuvant effect on the immune system. The effect of LAB on the immune system can be observed immediately after oral or parenteral administration. The effects on the immune system by oral intake of lactic acid-producing bacteria were evaluated by a clinical study carried out on healthy volunteers. The first group of healthy volunteers was given fermented dairy product containing *Lactobasillus acidophilus* strain La1, while the second group was given fermented dairy product containing *Bifidobacterium bifidum* strain Bb12 for 3 weeks. Blood samples were taken during the study and changes in lymphocyte subsets or leukocyte phagocytic activity were assessed immediately after intake of the fermented products. No change was noted for both groups in lymphocyte populations. However, *in vitro* study demonstrated that phagocytosis of *Escherichia coli* increased after the intake of all fermented products used. As a result of the continuous consumption of fermented products for 6 weeks, the fecal colonization of LAB and phagocytosis increased.

Nonspecific, antiinfective mechanisms of the immune system may be stimulated with specific LAB supplements. These strains can be given as dietary supplements to improve the immunological functions of certain age groups (born babies and elderly).

A previous study displayed that the intestinal immune system of young mice that were fed kefir enhanced, whereas there was no change in the immune sytem of elderly mice. In this study, 6-month-old (young) and 26-month-old (old) rats were fed standard kefir supplementation for 28 days, whereas control group rats received only standard diet. Cholera toxin (CT) was injected into mice intraduodenally on days 7 and 21. It was reported that anti-CT IgA concentration was increased in young rats that were fed kefir, while no change was observed in the elderly and control groups. Apart from these effects, the use of kefir may be recommended to protect the organism against the negative effects of radiation and to help repair the immune system.

Apart from these effects, the use of kefir may be recommended to protect the organism against the negative effects of radiation and to help repair the immune system (Thoreux and Schmucker 2001; Karatepe and Yalçın 2014).

7.4 Cholesterol-lowering Effect

On the basis of the various studies on cholesterol-lowering activity, cholesterol-lowering mechanism of some intestinal bacteria are explained as follows: cholesterol, which is synthesized in the body and taken with food, is transformed into bile acids. Some intestinal bacteria, such as *L. acidophilus*, are capable of deconjugating these formed bile acids. Deconjugated bile acids are more easily absorbed than lipids. In this way, the reduction in blood cholesterol level occurs.

Fermented dairy products obtained using *Lactococcus lactis* subsp. *lactis, L. lactis* subsp. *cremoris, Streptococcus lactis* subsp. *diacetylactis, Streptococcus salivarius* subsp. *thermophilus, Leuconostoc cremoris, Lactobacillus delbrueckii* subsp. *lactic, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus*, and *Saccharomyces cerevisiae* (without invertase) as starters cause a decrease in liver fat concentrations and blood cholesterol levels of mice that are fed high-cholesterol diet. It has been reported that these fermented dairy products significantly reduced total serum cholesterol and phospholipid levels in mice that were fed high-cholesterol diet. Human studies also showed that the fermented dairy products and their cultures have the cholesterol assimilation abilities (Tamai et al. 1996; Karatepe and Yalçın 2014).

7.5 Effects on Gastrointestinal System

7.5.1 Effects on Lactose Intolerance

Lactose is broken down into glucose and galactose by the enzyme lactase. Although depending on the ethnic origin, 15–80% of the adult human population has low lactase enzyme activity in the intestinal mucosa. As a result of bacterial fermentation of undigested lactose, some volatile compounds such as CO_2, methane, and hydrogen are released in the intestines. Kefir may be consumed easily by lactose-sensitive individuals because of the lower lactose content and increased lactase enzyme content. Many laboratory studies have displayed that lactose is more hydrolyzed in the intestine when fermented dairy products containing *Lactobacillus* are consumed.

In a clinical study, it was reported that complaints (abdominal pain, diarrhea etc.) from people with lactose intolerance increased when they consumed 500 ml of low-fat milk per day.

But, no such symptoms were observed when they consumed the same amount of yogurt, kefir, or acidophilus milk. For this reason, fermented dairy products may be included in diets formulated for persons with lactose intolerance (Zubillaga et al. 2001; Karatepe and Yalçın 2014).

7.5.2 Antimicrobial Effect

The beneficial bacteria (especially *Lactobacillus* ssp.) taken by the continuous consumption of kefir, settle in the intestines and correct the intestinal flora. The compounds in the acid structure produced by these beneficial bacteria inhibit the bacteria that cause disease.

In a previous study, it was observed that bacteria found in natural kefir microflora inhibited *Coliform* bacteria. When pathogenic bacteria, such as *Shigella* and *Salmonella* spp., together with the kefir starter were added to the milk, it was found that these pathogenic bacteria did not grow. Kefir shows antimicrobial effect against pathogenic microorganisms (*E. coli* and *Salmonella* ssp.) that cause diarrhea, thus preventing diarrhea. Owing to the presence of LAB and yeasts in microflora, kefir shows strong antimicrobial effect against pathogenic gut microorganisms.

It has been reported that the kefir has a synergistic effect with the gastric juice and can thus completely inhibit pathogens, especially *Salmonella typhimurium*, within 1 hour.

Today, cases of poisoning from *E. coli* O157:H7 are frequently encountered. It is thought that one of the effective and easy prevention methods against *E. coli*-induced intoxications is to provide colonization of a large number of *Bifidobacteria* and LAB in the intestine by consumption of yogurt and kefir (Ota 1999). In addition, the probiotic microorganisms in the kefir not only act on the intestines, but also some strains of these microorganisms can stimulate the body's immune system to protect against bacterial, fungal, or viral infections (Vrese and Schrezenmeir 2002; Karatepe and Yalçın 2014).

7.5.3 Effect on Helicobacter Pylori *Infection Treatment*

Fresh kefir has an effect that promotes the functioning of the stomach muscles and thus gastric emptying rate increases. In the treatment of *H. pylori*-induced ulcers, it was observed that the growth of *H. pylori* was prevented when alkaline kefir was used. On the other hand, it was also reported that gastric acidity decreased, gastric pain and digestive disorders improved with kefir consumption (Zubillaga et al. 2001; Karatepe and Yalçın 2014).

7.6 Antiallergic Properties

The incidence of food allergy, like other atopic disorders, also has increased in many people. Regular consumption of kefir milk and soy-based kefir is known to limit IgE- and IgG1-mediated responses in organism. Furthermore, the change in intestinal flora caused by kefir consumption may lead to the prevention of food allergy and to increase the mucosal resistance to gastro-intestinal pathogen infection. Another work showed that kefir inhibits ovalbumin-induced eosinophilia in lung tissue and mucus hypersecretion. In this way, kefir displays a potential therapeutic effect in the treatment of allergic bronchial asthma (Lee et al. 2007; Ahmed et al. 2013).

7.7 Antioxidant Effects

Dietary components play an important role in the protection of the body against oxidative damage. Kefir contains a lot of components with antioxidant activity. The researchers compared the effects of kefir and vitamin E on the protection of tissues against carbon tetrachloride-induced oxidative damage in animals. The results showed that kefir protects the tissues more than vitamin E against carbon tetrachloride-induced liver damage (Ahmed et al. 2013).

The results of the carbontetrachloride toxicity test on rodents exert that kefir has a higher antioxidant effect than vitamin E. In addition, the researchers evaluated the effect of kefir on oxidative stress induced by the use of lead. It is observed that 6 weeks after kefir consumption, glutathione peroxidase levels of the rodents increased. Malondialdehyde levels of animals consuming kefir exposed to lead poisoning were found to be similar to those of healthy animals.

These results support the hypothesis that kefir is a potential tool in the control of oxidative stress. In a study evaluating the antioxidant activity of kefir prepared with goat and cow's milk, it was reported that kefir inhibited linoleic acid peroxidation and possesses the 1,1-diphenyl-2-picrilhydrazyl and superoxide radical scavenging activity. It is known that there is a relationship between the amount of free radical production in the organism and increase in the risk of chronic disease. In addition, kefir stimulates the activity of antioxidant enzyme systems. Therefore, kefir consumption should be promoted as a natural defence method (Rosa et al. 2017).

7.8 Wound Healing Activity

Huseini et al. (2012) evaluated the antimicrobial and wound healing effects of kefir using the experimental burn wound model in rats. Burn damages were made on dorsal skin of rats. After 24 hours, burn damages also were infected with *Pseudomonas aeruginosa.* Kefir gels obtained from continuously cultured kefir grains in MRS Broth medium for 24, 48, and 96 hours together with the base gel, silver sulfadiazine ointment were applied on the infected burn wounds twice a day. The findings were compared with those of conventional silver sulfadiazine treatment. After 2 weeks of application, burn wound area, percentage of epithelization, scar formation, inflammation, and angiogenesis were evaluated. At the end of the 2nd week, the smallest wound size was observed in kefir 96-hour-gel-group animals. The fastest of wound epithelialization and scar formation and the lowest grade of inflammation also were observed in kefir 96-h-gel-group animals. As a conclusion, it was thought that the kefir gel treatment was an effective therapeutic approach to improve outcomes after severe burns as compared to silver sulfadiazine treatment (Huseini et al. 2012)

Bourrie, Willing, and Cotter (2016) compared the wound healing effects of neomycin-clostebol emulsion (5 mg/kg) with a gel prepared from kefir grains on open wounds inoculated with *Staphylococcus aureus* in rats. When the open wounds inoculated with *S. aureus* were treated with gel prepared from kefir grains, it was found that the wounds healed faster than the neomycin-clostebol emulsion. Furthermore, the gels obtained from kefir and kefir grains were found to be more effective in healing of third-degree burns contaminated with *Pseudomonas aeruginosa* as compared to rayanian sulphadiazine treatment. In addition to the burn wounds, in open skin wound model in the rabbit, it was found that the gel prepared from kefir was able to both heal wounds quickly and disinfect the infection. Reduced healing time is likely to depend on more than one factor. One of these factors may be due to the ability of kefir to inhibit bacterial and fungal growth. Another possible factor is the ability to modulate the immune system and use immune cells to aid the healing process (Bourrie, Willing, and Cotter 2016).

7.9 Antihypertensive Effect of Kefir

Some evidence suggests that probiotic bacteria or fermented products play an important role in controlling blood pressure. Antihypertensive effects of these fermented products have been observed in experimental and clinical studies, but the data are limited and controversial. By activation of bioactive peptides produced from casein during the fermentation of milk, it was found that the kefir could affect the angiotensin converting enzyme (ACE) activity. According to the researchers, the observed antihypertensive activity is associated with ACE inhibitory ability of kefiran. The ACE-inhibitory peptides inhibit the production of the vasoconstrictor angiotensin I. By ACE inhibition, the production of aldosterone hormone, which increases blood pressure, is inhibited. In addition, ACE-inhibitor peptides also inhibit the breakdown of bradykinin, a hormone with vasodilating effect, which contributes to blood pressure reduction (Rosa et al. 2017).

ACE-inhibitory activity was determined in kefir made from goat milk. This activity was due to the formation of low molecular weight peptides released from the main milk protein casein. In the fractions collected by the reverse phase HPLC method, the researchers identified 16 peptides. Two of these peptides (PYVRYL and LVYPFTGPPN) were found to have ACE-inhibitory activity. Researchers reported that kefir consumption significantly reduced blood pressure and also reduced the ACE activities in the thoracic aorta and serum of SHRSP/Hos rats. Serum total cholesterol, LDL-cholesterol, and triglyceride levels were also reported to be lower in the kefir group animals (Guzel-Seydim et al. 2011).

7.10 Protective Effect against Apoptosis

Exposure to UV rays causes the production of ROS in the skin, and the ROS damage the melanocytes together with the skin cells. Long-term exposure to UV causes the formation of freckles and wrinkles on the skin. In later periods, these benign formations on the skin may turn into skin cancer. Kefiran-kefir has superoxide radical scavenging activity, which provides protection against UV damage of human melanoma HMV-1 cell. In another study, researchers investigated the role of kefir extract in the apoptosis of HMV-1 cells caused by UV radiation. It was reported that kefir extract is able to repair DNA damage caused by UV radiation and to suppress apoptosis.

Also, it was determined that the apoptotic index of kefir-treated rats in the X-ray apoptosis model was significantly decreased (Matsuu et al. 2003). Kefir induces antiapoptotic effect for intestinal epithelial stem cells by inhibiting caspase-3 activity in rats. Hence, kefir treatment may reduce the side effects of radiation treatment in patients with malignant cancer (Ahmed et al. 2013).

8 Conclusion

Kefir, the traditional fermented milk product, is thought to be consumed primarily in the Caucasus Mountains, Tibet, and Mongolia. It is produced by the local people by using cow and goat's milk and is very refreshing. According to the Turkish Food Codex Communique on Fermented Milk Products, kefir is defined as a fermented milk product that uses starter cultures or kefir grains containing specific strains of *Lactobacillus, Leuconostoc, Lactococcus,* and *Acetobacter*; fermented lactose (*Kluyveromyces marxianus*); and nonfermented yeasts (*Saccharomyces unisporus, Saccharomyces cerevisiae* and *Saccharomyces exiguus*) for fermentation.

Kefir produced using the fermentation technique is one of the best sources of probiotics. Furthermore, this fermented food is also rich in minerals, protein, carbohydrates (especially kefiran), and vitamins that are beneficial for human health. Until today, scientific studies have been conducted on antimicrobial, antioxidant, antihypertensive, cholesterol-lowering, wound healing, and anticancer effects of kefir. In addition, kefir is found to have antiallergic and antiapoptotic properties, and also has positive effects on the immune system and gastrointestinal system.

In conclusion, kefir, which has many beneficial effects on health and is an important source of probiotics, should be recommended to be consumed frequently.

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16

Fermented Indigenous Indian Milk Products

Mohamed Yousuff Mohamed Imran
P.G. and Research Department of Microbiology and Biotechnology, National College (Autonomous), Tiruchirappalli, India

Nazar Reehana
P.G. and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli, India

Gangatharan Muralitharan, Nooruddin Thajuddin and Dharumadurai Dhanasekaran
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

Fermentation of Milk

Milk can be fermented by inoculation of fresh milk with appropriate bacteria and placing it at a temperature that favors bacterial growth. At the end of the incubation, milk sugar, lactose is converted to lactic acid, which can be experienced by a tart or sour taste. The reason behind this is that the lowered pH caused by lactic acid preserves the milk by preventing the growth of putrefactive and/or pathogenic bacteria that do not grow in acidic conditions.

Preparation of Fermented Milk Products

Fermented milk products are prepared from milk of almost all domesticated animals since ages. This traditional fermentation usually takes place as a result of activities of natural microbial flora either present in the food or added from the surroundings. Among the bacteria, the most important group carrying out the fermentation is lactic acid bacteria, which are generally regarded as safe for human consumption.

Fermented milk products, also called cultured milk products, are dairy foods that have been fermented with lactic acid bacteria. This fermentation process increases the shelf life of the product, while enhancing the taste and improving the digestibility of milk. Most important lactic acid bacterial group includes *Lactobacillus, Lactococcus,* and *Leuconostoc*. The term cultured milk products is used to designate fermented milks that are prepared by using starter cultures and predefined temperature.

During fermentation, certain physical and chemical changes take place in milk as a result of growth and fermentative activities of lactic acid bacteria. Cultured milk products are produced by using special lactic acid bacterial cultures, which fall under two broad categories.

1. Those made by using lactic acid bacteria and grow well at ambient temperature (25–30°C) are known as mesophilic starter cultures.
2. Those made by using lactic acid bacteria and grow well under higher temperature (38–45°C) are called as thermophilic starter cultures.

The flavor, texture, and consistency of the cultured dairy products vary depending on the type of cultures used. Cultured milk is also beneficial for the people who are lactose intolerant, those who have a deficiency of lactase enzyme, the enzyme that helps in breaking down lactose to glucose and galactose for further utilization. Consumption of cultured milk does not pose any problem for them because a large part of the milk sugar has already been digested by lactic acid bacteria.

The production of cultured milk products begins with good-quality milk and a good starter culture (Figure 16.1).

- The milk is first subjected to filtration to remove physical dirt.
- This is followed by pasteurization of the milk at 80–85ºC for 30 minutes in a boiling pasteurizer of 50 liter capacity.
- Once the pasteurization is complete, the milk should be cooled to 20–25ºC either in a cold water basin or a trough.
- Then the milk is inoculated with 2–3% active starter culture, stirred well for 3–5 minutes to ensure uniform distribution of starter culture, and then finally incubated at ambient temperature overnight (for 16–18 hours).

During the production, care should be taken for intact coagulation. This can be achieved by cooling the coagulants before stirring or breaking the curd. This may improve viscosity and consistency of the product.

Characteristics of Ideal Starter Cultures

1. It should be quick and steady in acid production.
2. It should produce the product with a balanced lactic flavor.

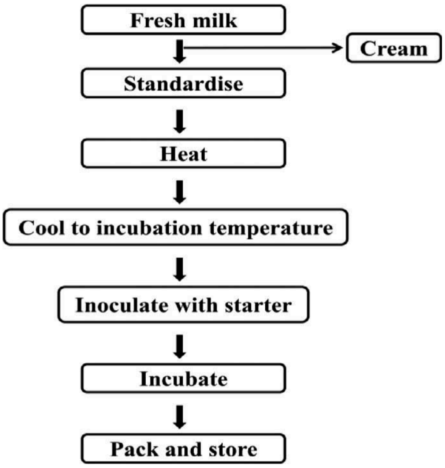

FIGURE 16.1 General steps involved in the preparation of fermented dairy products.

    a. It should not produce any pigments, gas, off-flavor, and bitterness in the finished products.
3. It should be associative in nature in product development.

Role of starter cultures in fermented milk

When starter cultures grow in milk, they affect the constituents of milk and bring fermentative metabolic changes. They produce different intermediary or end products, which gives typical characteristics to fermented milk such as

1. Production of lactic acid.
2. Bring about coagulation of protein and form gel.
    a. Produce volatile-flavored compounds such as diacetyl acetaldehyde and several intermediate compounds.
    b. Possess other compounds such as CO_2, alcohol, and propionic acid, which are essential in products like cheese.
3. Control the growth of pathogens and spoilage organisms.
    a. Some dietary cultures, such as *Lb. acidophiles*, have health benefits and produce antibacterial substances.
4. It helps in forming texture and ripening of cheese.

The primary function of starter culture is to develop desired amount of acidity in the products. The secondary effects of acid production include coagulation, expulsion of moisture, texture formation, and initiation of flavor production. In addition to these, the starters also help in imparting pleasant acidic taste confirming protection against potential pathogens and spoilage-causing microorganisms, and providing a longer shelf life to the product. Traditional fermented milk products are prepared from cow or buffalo milk or their combination. Table 16.1 shows various starter cultures used for manufacturing dahi, lassi, and shrikhand.

TABLE 16.1

Traditional Indian fermented milk products and starter cultures used for their preparation

Name of product	Country of origin	Type of milk used	Starter cultures used
Dahi	India, Persia	Cow or buffalo milk or their combination	*L. lactis* subsp. *lactis* *S. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus, plantarum* Lactose fermenting yeasts Mixed culture (not defined)
Shrikhand	India	Cow or buffalo milk or their combination	*S. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus*
Lassi	India	Cow or buffalo milk or their combination	*S. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus*

Dahi (curd)

Dahi is the local name for curd (Dewan and Tamang 2007). It is considered as a traditional fermented milk product and also a functional food as it is nutritionally and therapeutically beneficial for human beings (Yadav et al. 2007). Dahi is prepared by inoculating the heated milk (generally boiled milk) with starter culture. It is produced with varieties of taste, which varies from region to region as per the individual food habits. It is estimated that about 6.9% of total milk produced in India is utilized for making dahi.

Definition of dahi

Dahi or curd is a semi-solid product, obtained from pasteurized or boiled milk by souring it with harmless lactic acid or other bacterial cultures. It may contain additional cane sugar. It should have the same minimum percentage of fat and solids-not-fat (SNF) as the milk from which it is prepared (De 1980; Kumar and Mishra 2004).

Dahi made from buffalo milk is thick in consistency due to high content of SNF in the buffalo milk. In traditional method of dahi manufacturing, an increase of 10–15% in solids is observed, leading to thick consistency and desirable texture. It is recommended to make dahi from a mixture containing 11–13% SNF. SNF of milk can be increased by adding skim milk powder (SMP) or condensed skim milk. This resulting increase in protein content keeps the product from wheying off and provides a custard-like consistency (De 1980). Dahi prepared from whole milk contains about,

Fat : 5–8%
Protein : 3.2–3.4%
Lactose : 4.6–5.2%
Ash : 0.70–0.72%
Lactic acid : titratable acidity 0.60–0.80 %

Fat content in dahi can be standardized as per the demand of the consumer ranging from full fat to no fat. Post fermentation heat treatment to dahi can be helpful for the extension of its shelf life, but it destroys the bacterial cultures and hence reduces the health beneficial properties. Thus, the Codex mandates to differentiate the labeling standards by live microorganisms and the heat-treated products. Such products should be labeled as “contains no active culture”, so that the consumers can make an informed choice.

Dahi has live cultures that nurture therapeutic and health-promoting properties along with nutritional benefits. The lactose in the milk is converted to lactic acid by the action of starter cultures and the lactic acid, used as a preservative for the milk, and the low pH (4.5–5.0) also inhibit the growth of harmful microorganisms, thus increasing the shelf life of the product (Sodini et al. 2002).

The therapeutic value of dahi is due to the fact that the concentration of lactic acid bacteria in the intestine obtained by the ingestion helps in digestion of food (Nahar et al. 2007). Also, during fermentation the milk protein can be partially degraded by the action of bacterial proteases into peptides, possessing various health benefits. The availability of calcium in dahi is also increased because of milk, which increases its nutritional value. In this process, proteins are broken down into biopeptides, possessing various health benefits (Nielsen et al. 2009).

Dahi produced on domestic level has undefined cultures, thus, difference in the texture and taste is often observed. With standardization of culture and production conditions, a defined product of consistent quality (sensory and textural) can be achieved. Probiotic dahi is also manufactured by including secondary organism such as *Streptococcus thermophilus* and *Lactobacillus acidophilus*. It is also influenced by the quality of milk, temperature, time, and storage conditions (Garg and Jain 1980; Coggins et al. 2010).

TABLE 16.2

FSSR (2011) and BIS Standards of dahi

Characteristics	FSSR(2011)	BIS
Acidity % lactic acid	-	0.6–0.8
Total plate count	Not more than 1,000,000/g	
Coliform count	10/g max	10/g max
Escherechia coli	Absent in 1 g	
Salmonella	Absent in 25 g	
Shigella	Absent in 25 g	
Stephylococcus aureus	Not more than 100/g	
Yeast and mold	100/g max	100/g max
Anaerobic spore	Absent in 1 g	
Listeria monocytogenes	Absent in 1 g	
Phosphatase test	Negative	
Other requirements	It should have the same minimum percentage of fat and SNF as the milk from which it is prepared. If no standards are declared then standards prescribed for dahi from buffalo milk shall apply	Dahi shall conform to the requirements of milk fat and MSNF, as laid down in FSSR (2011)

The Bureau of Indian Standards (BIS) specification for fermented milk products are based on the types of culture used in their preparation (Anon 1990). Mild dahi is made from mesophilic *Lactococci*. *Leuconostoc* may be used to adjust the flavor to buttery flavor. Sour dahi contains additional cultures from thermophillic group of bacteria. For manufacturing of dahi, different kinds of milk (i.e., cow, buffalo, goat) can be used. The composition of milk used to produce cultured dairy products is given in Table 16.2.

Microorganism(s) Involved

Streptococcus cremoris, *S. lactis*, *S. thermophilus*, *Lactobacillus bulgaricus*, *L. acidophilus*, *L. helveticus,* and *Lactobacillus cremoris.* Whether native fermentation or inoculum is added, a small amount of already fermented curd is used as a starter inoculum.

Starter Culture Used in Preparation of Dahi

Milk is the natural habitat of numerous lactic acid bacteria causing the natural souring of milk, held at bacterial growth temperature, and appropriate length of time. Lactic acid bacteria can enter the milk from various sources such as air, utensils, milking equipment, milchers, and cow feed. The soured milk gets undesirable texture and flavor, reducing its acceptability, and contamination of the product from extrinsic factors can cause serious health hazards. However, during the commercialization, starters containing specific genes, species, and strains of cultures are used to generate desired flavor, consistency, and texture (Chandan and Shahani 1995).

A starter culture contains food grade microorganisms that on culturing in milk produce predictable attributes. Bacteria that grow at ambient temperature are called mesophilic bacteria, whereas those grow at higher temperature are termed as thermophillic bacteria. Usually, mesophilic bacteria are used to prepare dahi. Table 16.1 lists lactic acid bacteria used as starter cultures in the industries for manufacturing of various Indian dairy products.

For commercial success of dahi, the culture should meet the rapid acid development, typical dahi flavor, consistency, and texture Exopolysaccharide-secreting strains are used to enhance the viscosity of the product, scale up the possibilities in various production conditions, including compatibility with variety and levels of ingredients used, fermentation time and temperatures, survival of culture viability during shelf life of dahi, possessing desirable health and probiotic properties and exhibiting survival in the human gastrointestinal tract for certain health attributes, minimum acid production during distribution and storage at 5–10°C until dahi is consumed.

Microbiological specifications of these cultures suggest that counts of mesophilic lactic, yeast and molds, coliform, anaerobic spore formers, and salt-tolerant *micrococci* should not exceed 10 cfu/g. *E. coli*, *S. faecium,* and coagulase-positive *Staphylococci* should be less than 1 cfu/g. Culture should be free from pathogenic microorganisms.

Classification of Dahi

Dahi is prepared in different varieties with region-specific tastes. It may be classified on the following basis.

1. Dahi for consumption.
2. Dahi for production of desi butter.
3. Dahi for preparation of chakka, shrikhand, and lassi.
4. Dahi prepared from whole milk, skim milk, standard milk, and special milk.
5. Dahi prepared with added sugar and fruits.

Optimum acidity of normal dahi is less than 0.7% lactic acid while acidity of sour dahi is more than 0.7% lactic acid. Dahi made from buffalo milk produces a thick consistency product because of its high total solids content. It is recommended to make dahi from the milk containing 11–13% total solids. Higher milk solids yield dahi with higher consistency and also keep the product from wheying off.

Method of Preparation

Household utensils are required for preparation of dahi. It is generally prepared using cow or buffalo's milk. It is a weak gel when prepared from cow milk. The gel is relatively firm when buffalo milk is used because of the higher total solids (16–19% compared with 12% for cow milk). Whole milk dahi has a creamy layer on top of the homogenous curd.

Methods of Manufacturing Dahi

Traditional Method

In traditional method, dahi is prepared at a small scale, either in the consumer's household or in the confectionary (Halwais) shop. Milk is heated intensively to boil for 5–10 minutes, cooled to room temperature and inoculated with previous day's curd or butter milk at the rate of 0.5–1.0%. Milk is then stirred and allowed to set undisturbed for about overnight. At the confectionary shops, the method used for the preparation of dahi is more or less the same except that the milk is

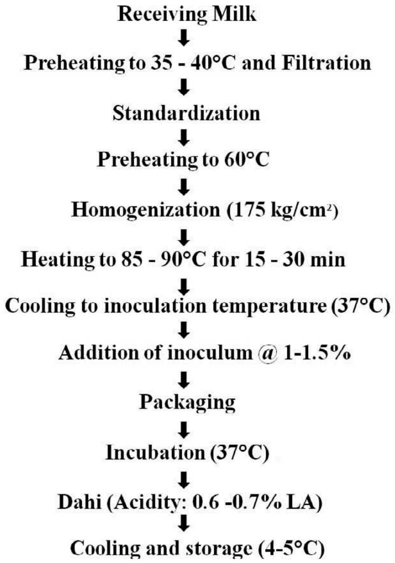
Receiving Milk
Preheating to 35 - 40°C and Filtration
Standardization
Preheating to 60°C
Homogenization (175 kg/cm²)
Heating to 85 - 90°C for 15 - 30 min
Cooling to inoculation temperature (37°C)
Addition of inoculum @ 1-1.5%
Packaging
Incubation (37°C)
Dahi (Acidity: 0.6 -0.7% LA)
Cooling and storage (4-5°C)

FIGURE 16.2 Flow chart for manufacturing dahi.

concentrated in open pan before inoculation. Concentration of milk results in custard-like consistency of dahi and keeps the product from wheying off.

Industrial Method of Dahi Making

Organized sector produces dahi on the basis of scientific lines. Fresh, sweet, good-quality milk is received, preheated to 35–40°C, and subjected to filtration and clarification to ensure that the milk is free from extraneous matter. The milk is standardized to 2.5–3.0% fat and 10–12% SNF, preheated to 60°C, and homogenized at a pressure of 175 kg/cm^2 in single stage. The milk is then heated to 85–90°C for 15–30 minutes, cooled to 37°C and inoculated with 1–1.5% of specific dahi starter culture. It is then filled in suitable packaging containers (food grade polystyrene and polypropylene cups) of the appropriate size and incubated at 37°C for suitable time. After proper setting of the dahi, its acidity reaches 0.6 to 0.7% and a firm curd is formed. The product mix is incubated till its pH reaches 4.4–4.5 and then it is cooled rapidly to less than 5°C by exposing the cups to high-velocity cold air. Dahi is normally stored at 4–5°C. Storage area should be maintained clean and tidy to avoid any cross-contamination. The flow diagram for manufacture of dahi is presented in Figure 16.2.

Technology of Dahi Manufacture

Dahi is made from fat-standardized milk. All ingredients should be of high quality and should maintain the quality standard. While manufacturing dahi at commercial level it is necessary to standardize and control the day-to-day product to meet consumer expectations and regulatory obligations. As the total solids in cows and buffaloes milk ranges from 11 to 17%, therefore the cultured products from these milks vary in consistency from fluid to custard like. Casein also plays an important role in determining the texture of dahi, casein coagulates on souring at isoelectric pH 4.6. The whey proteins are considerably denatured by heat treatment prior to culturing and as the denatured whey protein reacts with kappa casein (k-casein) as well as with other whey proteins, this ensures a better network formation and a firmer gel.

Equipment Used in Dahi Manufacture

A cultured milk plant should be specially designed to minimize the contamination of product with pathogenic microorganisms. Even air is filtered in this regard. This type of plant has separate receiving section to receive, weigh, and store the raw material. The culture room with control laboratory is completely separated from the processing section. In addition, a dry storage area, a refrigerated storage area, a mix processing room, a fermentation room, and a packaging room are maintained in the plant. A mix processing room containing equipment for standardization, milk separation, pasteurizing, incubation, heating, and homogenization along with the necessary pipelines, fittings, valves, and controls is also established (Bhattacharya et al. 1972; De Brabandere and De Baerdemaeker 1999; Soukoilis et al., 2007).

Milk is generally stored in silos. The inner layers of silos are made up of good-quality stainless steel (Grade AISI 304) to avoid corrosion. The stainless steel is then covered with 125 mm of insulated materials, which in turn are surrounded by outer shell of stainless steel or aluminum. For processing dahi and other fermented products, the mixture is heat treated more severely (between 80 and 85°C) than the conventional pasteurization methods. There are many beneficial effects of these heat treatments such as,

1. development of sterile medium that inhibits the growth of any undesirable microorganism.
2. removal of air from the medium to produce a more conductive medium for the growth of microaerophilic lactic cultures.
3. effecting thermal breakdown of milk constituents, especially proteins that release peptones and sulfhydryl groups which provide nutrition and anaerobic conditions of the starter culture.
4. it denatures and coagulates the milk protein, which enhance the viscosity of the product (Bhattacharya et al. 1972).

Homogenization

The homogenizer is a high-pressure pump forcing the mix through extremely small orifice. Homogenization is generally conducted by applying pressure in two stages. The pressure in the first stage is of approximately 210 kg/cm^2, which reduces the average particle size of fat to approximately 4 microns of diameter. The second stage uses 35 kg/cm^2 pressure, used to break the clusters of fat globules separately. After homogenization, all the fat globules of the milk have an average size below 1 micron. Homogenization of milk reduces the surface creaming in dahi and ensures a better texture due to the fact that the increase in total surface area of the fat globules resulting from homogenization results in milk proteins (primarily casein) absorbing onto the surface of the fat globules. The homogenized fat globules thus act as active components of the protein network in the final dahi (Bhattacharya et al. 1972; Wu et al. 2000).

Fermentation

Fermentation starts with the addition of culture in the milk. Addition of culture is done after cooling the milk to 37°C. The inoculated milk is then packaged in separate cups with lids. After that the cups are arranged in crates, which are then transferred to hot room (37°C) while the fermentation process proceeds. The pH of the milk in the cups should be regularly checked and when it reaches 4.4–4.5, these crates are transferred to room with temperature 3–4°C for proper setting. Figure 16.1 shows the process flowchart for manufacturing dahi.

Dahi normally contains no added sugar or flavor. Consumers can add sugar and flavor in to it as per their choice. It can also be used for dressing of salads of fresh fruits and grated vegetables (De 1980; Routray and Mishra 2011).

Packaging and Storage of Dahi

Dahi is generally packaged in polyethylene, polypropylene, polystyrene packaging material, and plastic cups (Saint-Eve et al. 2008). It is recommended to store the packaged dahi in between 1 and 4 °C to extend its shelf life. At 7°C, the shelf life of dahi is estimated to be 2–3 weeks.

Quality Control for Dahi Manufacture

To make a product with the most desirable attributes, the standards of fats, solids, viscosity, pH, and organoleptic characteristics should be strictly followed. In the case of dahi, yeast is more potent contaminant than molds or bacteria. Yeast and mold contamination may arise by contaminated starter cultures, packaging materials, and equipment. This could be avoided by following good sanitation processes. Organoleptic examination and microscopic examination of the starter could be helpful in eliminating the fungal contamination. Contaminated cultures should be avoided for product manufacturing. Lactic acid and some other metabolic products that are formed during the fermentation process inhibit the growth of most of the Gram-negative psychotropic bacteria (Anon 1990).

Symptom of Quality Product

A good-quality dahi is of firm and uniform consistency with a sweet aroma and clean acid taste. The surface is smooth and glossy and a cut surface is trim and free from cracks and gas bubbles. The composition of dahi depends upon the type of milk used and the manufacturing conditions.

Shrikhand

Shrikhand is a semi-soft, sweetish sour, whole-milk product prepared from fermented curd (Kulkarni et al. 2006). The curd (dahi) is partially strained through a cloth to remove the whey to produce a solid mass called chakka (the basic ingredient for shrikhand). Chakka is mixed with the required amount of sugar to yield shrikhand (Aneja et al. 1977). The product thus obtained is garnished with nuts and condiments. Shrikhand is a very popular product and forms the part of meal on special and festive occasions particularly in the states of Gujarat and Maharashtra (Jain et al. 1998). Like dahi, it is also a very refreshing product, particularly during summer season. The composition of shrikhand is moisture 34.48–35.66%, fat 1.93–5.56%, protein 5.33–6.13%, reducing sugar 1.56–2.18%, and nonreducing sugar (sucrose) 55.55–53.76%.

Addition of artificial coloring or flavoring substances in shrikhand is discouraged (Patel and Chakraborty 1988). According to PFA, shrikhand is the product obtained from whole or skimmed milk chakka, to which milk fat is added (Upadhyay and Dave 1977). It may contain fruits, nuts, sugar, cardamom, saffron, and other condiments. Any added coloring and artificial flavoring substances should not be used.

To further extend the shelf life of shrikhand, a preparation known as shrikhand vadi, which is essentially a desiccated shrikhand, is made. The dewatered dahi is mixed with an equal amount of sugar by weight and dried in an open pan at low heat. When the mass begins to harden, it is tested for stickiness. The nonsticky product is flavored and colored. Powdered sugar is further added as desired. The product is mixed, rolled, cut into shapes, and packed like biscuits. The product yield is about 50% of the original weight of milk. The composition of shrikhand vadi is moisture 6.5%, fat 7.4%, protein 7.7%, ash 0.8%, reducing sugar 15.9%, and nonreducing sugar 68.9% (Date and Bhatia 1955).

Technology of Manufacturing Shrikhand

The manufacturing of shrikhand consists of preparation of curd by adding starter culture to milk, preparation of chakka by draining the whey, and mixing of additional ingredients such as sugar and condiments to obtain the desired product.

Traditional Method of Shrikhand Production

Traditionally, cow or buffalo milk is heated to near-boiling temperature and then cooled to 30–35°C. It is then inoculated with lactic acid bacteria culture at the rate 0.5–1% of milk. Milk is then left at room temperature (30–35°C) for 5–7 hours for setting of curd (Patel and Chakraborty 1985a). When dahi is firmly set, it is hung in muslin cloth overnight to drain out the whey. After 10–12 hours, chakka is obtained. It has been observed that there is 65% recovery of chakka from milk depending on the composition of milk (Patel and Chakraborty 1985b). Then

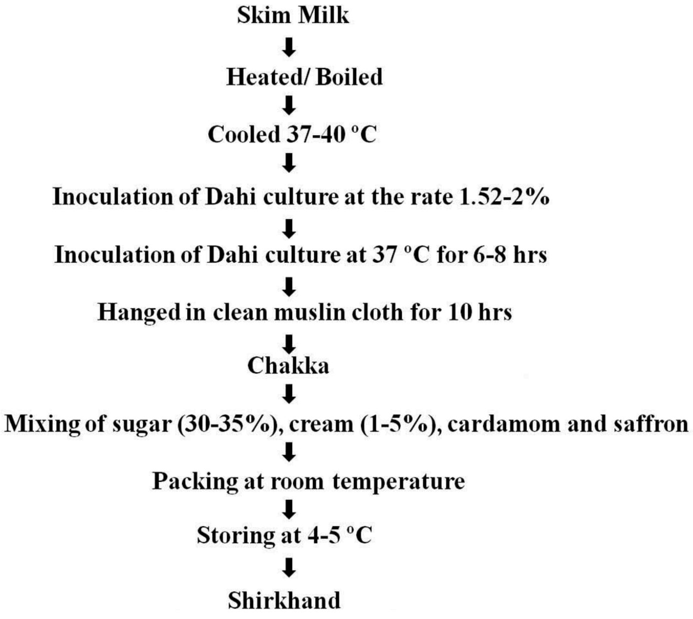
Skim Milk
Heated/ Boiled
Cooled 37-40 °C
Inoculation of Dahi culture at the rate 1.52-2%
Inoculation of Dahi culture at 37 °C for 6-8 hrs
Hanged in clean muslin cloth for 10 hrs
Chakka
Mixing of sugar (30-35%), cream (1-5%), cardamom and saffron
Packing at room temperature
Storing at 4-5 °C
Shirkhand

FIGURE 16.3 Flowchart for manufacturing Shrikhand.

chakka is mixed with the required amount of sugar (usually 50–100% of chakka weight), condiments, and other herbs. Commonly used spices or condiments are cardamom, saffron, cream, charoli (*Buchanania lanzan* is a tree that produces the seeds known as charoli, also called chironji). These seeds are used as a cooking spice primarily in India. Charoli are tiny almond-flavored dried seeds of a bush. After the hard shell is cracked, the stubby seed within is as soft as a pine nut, almonds, nutmeg, cashew nut, mango pulp, raisins, and seasonal fruits (Bhattacharya et al. 1971). About 1.5–2 kg of shrikhand is obtained from 1 kg of Chakka (Sharma 1998). Shrikhand is served chilled. The flowchart for manufacturing shrikhand is shown in Figure 16.3. In Gujarat, people prefer sweetened shrikhand, whereas in Maharashtra, salty or sour shrikhand is preferred (Jain et al. 1998).

Industrial Method

Mechanization of Manufacturing of Shrikhand

A fully mechanized and continuous process has been developed for manufacturing of shrikhand (Patel 1997). The first modern plant for manufacturing shrikhand was established at Baroda District Cooperative Milk producers Union Ltd. (Sagun unit) in Gujarat in 1980s, under the brand name of Sagun and Amul. The industrial production involves the following steps:

Preparation of Curd

Skim milk with 9% SNF, 0.05% fat is heated to near-boiling temperature and then cooled to 30–35°C (Patel and Chakraborty 1985c; Singh et al. 2014). Then dahi culture at the rate 0.5–1% is added with constant stirring. After 8 hours of incubation, the required acidity (0.8–1%) is achieved. The well-set curd can be used for chakka manufacturing. The dahi used for the preparation of shrikhand is inoculated with mixed culture containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. diaacetylactis/*Leuconostoc*, *Lactococcus lactis* subsp. *cremoris* in equal amounts (Chandan and Shahani 1982). Chakka is manufactured by draining the whey from curd. Draining of whey from curd is done mechanically, using either a basket centrifuge at 1100 rpm or a quarg separator.

To mix the ingredients, planetary mixers of capacity of only 40 kg/batch were earlier used. However, now scraped surface heat exchangers (SSHE) are preferred over the traditional mixers, since these have the capacity of mixing of more than 500 kg per minute. These types of mixers in combination of two or three can be used in a continuous production system (Aneja and Vyas 1983a).

Shrikhand is prepared by adding sugar and other desired ingredients with chakka (Aneja and Vyas 1983b). Sugar is added at the rate 50–100% of chakka weight and is mixed in SSHE. Some amount of cream is also added to attain the final fat in the product to 8.5% on dry matter basis (Patel 1997). The titratable acidity should be in the range of 1.10–1.40% LA. Herbal shrikhand is manufactured by adding water extract of Brahmi and Ashwagandha (Brahmi and Ashwagandha are Indian origin herbs that have large amount of antioxidant and polyphenolic components. Brahmi has memory-enhancing properties while Ashwagandha has immunomodulatory, anticancerous, and health-promoting benefits) to chakka along with other ingredients that possess many antioxidant properties and different medicinal properties such as immunostimulatory, memory enhancing, and antihypertensive (Prasad 2011). Guava pulp-enriched shrikhand was manufactured by Kumar (2011), to increase the amount of vitamin C in the shrikhand. This product has been found to be organoleptically acceptable. The product was slightly creamy in color than the conventional shrikhand. For packaging, generally preformed polystyrene cups are used (Patel and Chakraborty 1985b).

The shelf life of shrikhand depends on the temperature and initial microbial load of the raw materials (Sharma and Zariwala 1980). At ambient temperature, i.e., at 30°C it gets spoiled within 2–3 days; but under refrigerated condition (4°C), it may be kept for 40 days. Shrikhand is stored at −18 to −20°C, which enhances its shelf life for 6 months (Aneja and Vyas 1987).

Physicochemical Properties of Shrikhand

Different manufacturers make shrikhand as per the demands of their consumers. There is a little bit variation in the chemical and physical properties of shrikhand that is manufactured in Gujarat and Maharashtra (Jain et al. 1998). Variations can be found in shrikhand that is available in the market under different brands.

Chakka Powder

For preparation of chakka powder (Figure 16.4), first of all, whey is removed from dahi, and then dahi is ground in a colloid mill to obtain slurry with smooth and uniform consistency. The slurry is then heated to 38°C followed by homogenization at a pressure of 100 kg/cm^2. Finally, the slurry is passed through a spray drier with inlet temperature 185°C and outlet temperature 85°C to obtain chakka powder (De and Patel 1989). Chakka prepared from whole milk is considered as the superior powder on the basis of its consistency and aroma for incorporation in shrikhand.

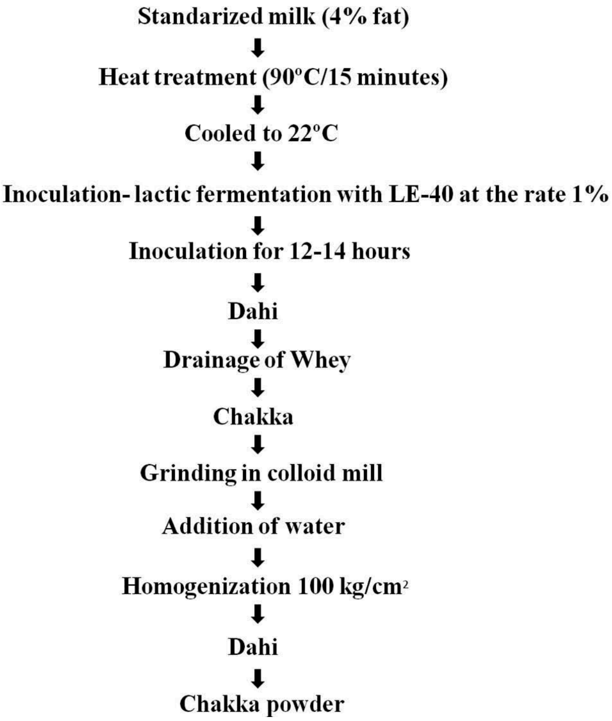
Standarized milk (4% fat)
Heat treatment (90°C/15 minutes)
Cooled to 22°C
Inoculation- lactic fermentation with LE-40 at the rate 1%
Inoculation for 12-14 hours
Dahi
Drainage of Whey
Chakka
Grinding in colloid mill
Addition of water
Homogenization 100 kg/cm2
Dahi
Chakka powder

FIGURE 16.4 Flowchart for the manufacturing of standardized milk chakka powder.

Lassi

Lassi is the local name of buttermilk. Lassi, also called stirred dahi, is a refreshing summer beverage, mainly popular in north India. Lassi is white to creamy white in color, liquid viscous, with sweet taste, rich aroma, and slightly acidic. Depending upon the consumer preference, lassi can be flavored with either salt or sugar and other condiments or spices. Lassi is manufactured using pasteurized milk cultured with flavor-producing culture microorganisms (Aneja et al. 1989). It is estimated that approximately 2144 million kg of lassi is produced in India annually.

The composition of lassi varies considerably. The factors affecting its composition are the type of milk used, extent of dilution during churning, and efficiency of fat removal. The composition of lassi is given below according to (Rangappa and Achaya 1974),

Water : 96.2%
Fat : 0.8%
Protein : 1.29%
Lactose : 1.2%
Lactic acid : 0.44%
Ash : 0.4%
Calcium : 0.6%
Phosphorus : 0.04%

The phospholipid-rich fraction obtained as a by-product during churning of dahi while making makkhan (fresh butter) may also be termed as lassi. Lassi is originally a product of rural India and was earlier only produced at cottage scale. However, commercial production of this product is now established. The characteristic sulfur flavor imparted to the product could be masked by the addition of 0.07–0.09% crushed green ginger and 0.5–0.7% salt. Salted lassi is marketed in many parts of the country. Commercially available lassi can be aseptically packaged in milk cartons/sachets/UHT cartons (Ramana 1994; Antunes et al. 2007).

Technology for Manufacturing Lassi

Newer technologies have been incorporated in the manufacturing of lassi. Pasteurization, homogenization, and culturing system are the steps that are used during the manufacturing processes (Tiwari 1998). Traditionally, lassi was manufactured by stirring dahi with small amount of water. Other additives in it depend on the local preferences. In northern part of India, lassi is mixed with sugar while in southern part of the country, salt along with other condiments such as crushed ginger, green chillies, and curry leaves are mixed in it. In the southern states of India, lassi is preferred less viscous as compared to northern parts, and has a pleasant aroma. Commercially, lassi is prepared by churning dahi mechanically and then adding sugar in the form of syrup. Syrup is added to improve the thickening of lassi and also to eliminate the addition of water. To improve the texture, homogenization is applied. Lassi can be flavored with rose or kewara (it is a shrub with fragrant flowers. They are used to extract perfume, aromatic oil (Kewara oil) and fragrant distillations. These are stimulant and antispasmodic and are used against headache and rheumatism. The flowers are also used to flavor food. Kewara essence is used in numerous Indian sweets like rasogolla, petha (candied gourd or winter squash), rasmalai and burfi (especially in Rajasthan)) essence and then packaged in bottles or pouches and stored at refrigerated temperature. The flowchart showing manufacturing process of lassi is shown in Figure 16.5.

Under refrigerated condition, the shelf life of lassi can be enhanced. Further extension can be achieved by UHT processing after fermentation and aseptic packaging (Ramana 1994). Industrial

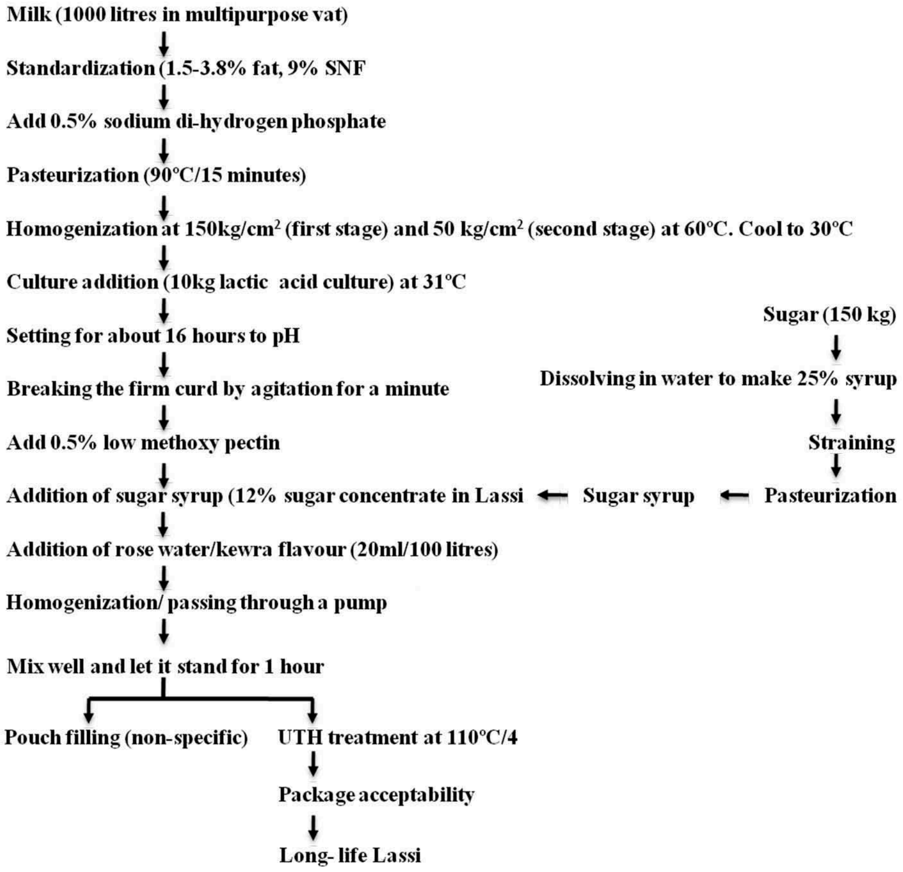
Milk (1000 litres in multipurpose vat)
Standardization (1.5-3.8% fat, 9% SNF
Add 0.5% sodium di-hydrogen phosphate
Pasteurization (90°C/15 minutes)
Homogenization at 150kg/cm² (first stage) and 50 kg/cm² (second stage) at 60°C. Cool to 30°C
Culture addition (10kg lactic acid culture) at 31°C
Setting for about 16 hours to pH
Breaking the firm curd by agitation for a minute
Add 0.5% low methoxy pectin
Addition of sugar syrup (12% sugar concentrate in Lassi
Sugar (150 kg)
Dissolving in water to make 25% syrup
Straining
Pasteurization
Sugar syrup
Addition of rose water/kewra flavour (20ml/100 litres)
Homogenization/ passing through a pump
Mix well and let it stand for 1 hour
Pouch filling (non-specific)
UTH treatment at 110°C/4
Package acceptability
Long- life Lassi

FIGURE 16.5 Flowchart for mechanized production of lassi.

production of lassi has made significant advancements through application of UHT processing. Standardized milk (9–10% SNF, 0.5–1.0% milk fat) is heated at 85°C for 30 minutes or 91°C for 2.5–5 minutes, and then suitable lactic acid culture is inoculated at 31°C (Chandan and Shahani 1995). It is then allowed to ferment by keeping the milk at 22°C till its pH reduces to 4.5. Then the set curd is stirred broken with mechanical stirrer along with mixing of sugar syrup (30%). This gives 8–12% sugar concentration in the blend. For proper texture, lassi is homogenized at 13.7 Kpa and UHT-processed at 135–145 °C for 1–5 seconds, and then packaged aseptically by using suitable equipment (Ladkani et al. 1993). The problems in the quality of lassi are very much similar to those of dahi.

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Section 6

Fermented Beverages

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17

Kvass: A Fermented Traditional Beverage

Hasya Nazlı Ekin and Didem Deliorman Orhan
Department of Pharmacognosy, Faculty of Pharmacy, Gazi University,
Ankara, Turkey

1 Introduction

Kvass is a beverage produced from rye bread, rye malt, rye flour, barley malt, or barley flour by using commercial or traditional fermentation techniques. It is a traditional beverage consumed especially in Russia and other Eastern European countries (Marsh et al. 2014; Jargin 2009). For many years, kvass has been mixed with cold soups called “Okroshka” and “Botvinya” in Russia. The first known recording about this fermented beverage dates to the year 989, when the Russians accepted Christianity.

Kvass has many medicinal properties (antiviral, antioxidant, antiinflammatory, and anticancer activity) as well as beneficial effects on health (Lidums, Karklina, and Kirse 2016). Kvass usually contains “lactic acid bacteria” such as *Lactobacillus casei* ATCC 334, *L. casei* FUA 3087. Therefore, it is a probiotic beverage that can be consumed by people with lactose intolerance. Since kvass contains more than ten amino acids, many B-complex vitamins, and minerals (manganese, magnesium, selenium, copper, and iron), its nutritional value is extremely high. Bread yeast used in fermentation influences the aroma composition of kvass. It is a foaming beverage with bitter-sweet taste and brown-yellow color (Lidums et al. 2015; Gambus et al. 2015).

The other type of kvass, which is obtained by fermenting slice beetroot (*Beta vulgaris* L.), is a traditionally consumed beverage in Poland. This kvass is known as “beet kvass” and has therapeutic effects such as antioxidant and antiinflammatory activities. This fermented beverage is rich in minerals, betalains, oxalic acid, phenolic acids, and sugars (Jakubowski 2017).

2 Rye Bread Kvass

2.1 Preparation

2.1.1 Traditional Preparation

For the production of kvass, the dried rye bread is extracted with hot water and incubated for 12 hours at room temperature. After bread yeast and sugar are added to aqueous extract, the mixture is fermented for 12 hours at 20°C (Lidums et al. 2015; Dlusskaya et al. 2008).

In another method used for production of kvass, rye malt is added to rye flour boiled with water and mixed. Bread yeast and sugar are added into this mixture (saccharification process) and then fermentation is initiated by adding starter (baker’s yeast). The mixture is fermented for 12 hours at 20°C (Dlusskaya et al. 2008). *Juniperus communis* L. berries, raisins, cranberries, mint, honey, lemon peel, essential oils etc. can be added to kvass for giving aroma and for various other purposes (Gambus et al. 2015; Costa et al. 2013; Baschali et al. 2017).

2.1.2 Industrial Preparation of Kvass

Kvass is produced industrially in recent years. The rusks of rye bread, rye flour, or defatted corn flour used as carbohydrate sources together with rye bread malt (freshly germinated or kilned) used for odor and acidity are mixed and moisturized with hot water. After 3 hours, the mixture is filtered, concentrated, and heated at about 100–130°C for 30 minutes. The concentrated mixture is used for the production of kvass beverage. Then, *Saccharomyces cerevisiae, Leuconostoc mesentericus,* and sugar are added and the mixture is fermented up to an alcohol content of 1.2%, followed by carbonation and bottling of the mixture (Behrmann et al. 2013; Lidums et al. 2014).

2.2 Microflora and Chemical Properties

The naturally produced kvass is fermented with *Saccharomyces cerevisiae* (bread yeast) (Dlusskaya et al. 2008). Lidums, Karklina, and Kirse (2016) compared the amounts of the dry matter in kvass samples fermented with *Kluyveromyces marxianus* and *S. cerevisiae.* As a result, the amount of dry matter (8.6%) in kvass fermented with *S. cerevisiae* was found to be higher than that of kvass fermented with *K. marxianus* (7.0%).

Lactobacillus casei ATCC 334, *L. casei* FUA 3087, *Leuconostoc mesenteroides* spp. *dextranicum* CECT 912^T, *L. mesenteroides* spp. *mesenteroides* DSM 20343^T, and *L. mesenteroides* FUA 3086 are probiotic bacteria present in kvass (Dlusskaya et al. 2008).

Lidums and Karklina (2014) investigated the time-dependent change of the microbial composition of kvass. During the 12-hour fermentation process, the yeast concentration raised from 4.55 log cfu/g to 6.06 log cfu/g. The amount of lactic acid bacteria increased faster than yeast microorganisms and total plate count in first 12 hours. After 130 hours of storage, the amount of microorganisms in kvass began to decrease.

The acidity of kvass rises with increased lactic acid concentration. The change in acidity causes the proliferation of lactic acid and yeast bacteria, and a decrease in the number of saprophytic bacteria (Lidums and Karklina 2014).

After kvass production, various microorganisms such as *Escherichia coli, Salmonella,* and *Staphylococus aureus* can cause the deterioration of its qualitative and quantitative properties. *Acinotobacter*sp., *Moraxella* sp. and *Pseudomonas* sp. may lead to an alteration in taste or odor of kvass. Additionally, kvass contaminated with *Clostridium botulinum* and *C. perfringens* may pose a threat to human health (Lidums and Karklina 2014).

In the GOST 28538-90 standard of Russia, the quality parameters of the commercial kvass are specified. According to this reference standard, the acidity of kvass must be in the range of 16–40 cm^3 1 M NaOH solution per 100 g concentrate. On the other hand, its dry matter content must be 70.0% in the commercially concentrated kvass (Lidums, Karklina, and Kirse 2016).

2.3 Nutritional Values

Kvass contains 1–1.5% or less alcohol (Baschali et al. 2017; Jargin 2009; Lidums et al. 2015). Lidums et al. (2015) investigated the nutritional values of kvass that was produced naturally. In this study, protein, fat, and carbohydrate contents of kvass were reported as 0.15±0.02, <0.10, and 5.90±0.02 g/100g, respectively. Its energy value was found as 130.40 kJ/100 g. Thiamine (B_1), Riboflavin (B_2), and Niacin (B_3) content of kvass were also determined as 0.71±0.09, 1.28±0.12, and 18.14±0.48 mg/100 g, respectively. Main volatile compounds in kvass were 4-penten-2-ol followed by carvone, ethyl octanoate, 3-methyl-1-butanol, ethyl decanoate, 2-phenylethyl acetate, isoamyl acetate, benzyl alcohol, hexanal, acetic acid, furfural, decanoic acid, furfuryl alcohol, hexanoic acid, phenylethyl alcohol, octanoic acid, ethyl caproate, hexyl acetate, and heptyl acetate (Lidums et al. 2015).

The amounts of glucose, fructose, maltose, and sucrose in kvass were determined as 77.60, 92.20, 7.97, and 29.52 g/L, respectively (Lidums, Karklina, and Kirse 2016). The mineral content of kvass also was investigated and the amounts of manganese were determined as 40 mg, 8–25 mg, <7 mg, 3–10 mg, 0.2–0.5 mg, <0.06 mg, <7 mg, 5–16 μg, <0.2 mg, 0.06–0.2 mg per 100 g, respectively (Albuquerque et al. 2013).

2.4 Biological Effect

It is stated that kvass is effective in relieving stomach disorders such as hyperacidity and flatulence, because it contains lactic acid which is an antioxidant and increases various minerals like calcium (Gambus et al. 2015).

2.5 Storage and Shelf Life

The shelf life of naturally fermented kvass is short, because it contains small amounts of alcohol and this amount is ineffective in protecting the stability of kvass (Lidums et al. 2015). The shelf life of kvass produced commercially is 8–12 months when stored at −40°C to 30°C, whereas this period is about 5 days for naturally fermented kvass (125 ± 5 hours) (Lidums et al. 2014; 2016).

3 Beetroot Kvass

3.1 Preparation

For the preparation of beetroot kvass, two or three beetroots are peeled and chopped. These chopped beetroots together with 1 tablespoon salt and 1/4 cup whey are placed in 2-quart glass container. The container is filled with water, stirred, and closed. The mixture is kept at room temperature for 2 days then is kept in refrigerator (Fallon et al. 2001).

3.2 Microflora and Chemical Properties

Lactobacillus acidophilus LA 39, *Lactobacillus casei* A4, *Lactobacillus delbrueckii* D7, and *Lactobacillus plantarum* C3 are used for fermentation of beetroot juice, but *L. acidophilus* and *L. plantarum* produce higher amounts of lactic acid. It was determined that the pH of fermented beetroot juice was less than 4.5 and it contained significant number of lactic acid bacteria (10^9 cfu/mL). *L. acidophilus* in the fermented beetroot juice was found less stable than other cultures (*L. casei*, *L. plantarum,* and *L. delbrueckii*) at 4°C (Yoon et al. 2005).

Beta vulgaris is one of the most important plants that include betalanin. Betalanins are water-soluble pigments, which are stable in acidic pH (pH 3–7) and include betaxanthins and betacyanins. Betacyanins are pigments with red-violet color. Betanin, betanidin, isobetanin, isobetanidin, and neobetanin are betacyanins; vulgaxanthin I and vulgaxanthin II are betaxanthins. Betanin is one of the betalanins that is found in the beetroot and can exhibit good bioavailability. Betacyanins have free radical scavenging effects and play an effective role in reducing oxidative stress due to these properties. Their biological activities and nutritional values can diminish with storage. Lactic acid fermentation can protect biological active components of kvass. After fermentation, betanin and isobetanin convert to their aglycones betanidin and isobetanidin (Strack et al. 2003; Klewicka et al. 2015).

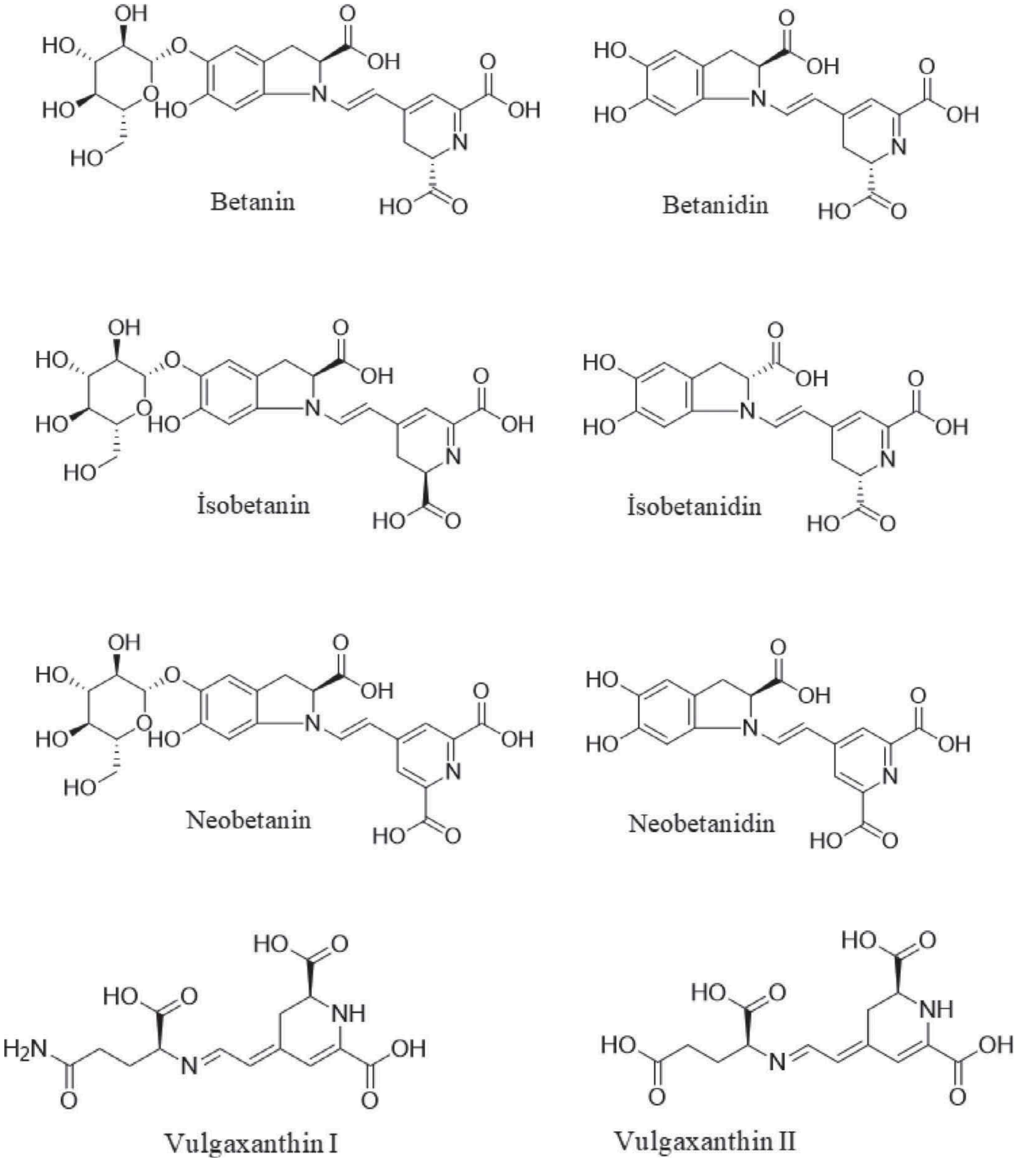

FIGURE 17.1 The structure of betalanins in *Beta vulgaris.*

Beta vulgaris (beetroot) also contains flavonoids, carotenoids, folates, saponins, and polyphenols such as phenolic acids and phenolic amides (Clifford et al. 2015; Kujala et al. 2002; Jastrebova et al. 2003; Ramos et al. 2017).

The total polyphenolic content of beetroot juices, which were fermented with *L. acidophilus* NCIM 2903 and *L. plantarum* VITES07 (HF571129) for 24 and 48 hours, was investigated by Vaithilingam et al. (2016). The concentrations of polyphenol samples fermented for 24, 48 hours, and raw beetroot juice were found to be 0.023, 0.104, and 0.011 mg/mL, respectively. When compared with raw and 24-hour fermented beetroot juice, the polyphenol contents were found to be more in 48-hour fermented juice.

3.3 Nutritional Values

The nutritional values of beetroot juice, which was fermented with brewer's yeast auto lysate in a 1:1 ratio and was inoculated with *Lactobacillus acidophilus* NCDO1748, were determined in a study conducted by Rakin et al. (2007). The protein, carbohydrate, lipid, and ash contents were determined as 4.05 ± 0.03, 4.13 ± 0.07, 1.32 ± 0.03, and 0.33 ± 0.02 g/100 mL, respectively. The amounts of Ca, Mg, Na, K, Fe, and P in the fermented beetroot juice were detected as 0.180 ± 0.01, 0.236 ± 0.02, 0.625 ± 0.04, 1.88 ± 0.06, 7.22 ± 0.03, and 0.595 ± 0.03 g/L, respectively. The percentage of betanin was calculated as 0.384 ± 0.05. The amount of vitamin B_1, B_2, B_6, C, and β-carotene was determined as 1.77 ± 0.09, 1840 ± 20, 0.26 ± 0.02, 103 ± 1.05, and 1.30 ± 0.03 mg/L, respectively (Rakin et al. 2007).

3.4 Biological Effect

Beetroot juice fermented with *Lactobacillus brevis* 0944 and *Lactobacillus casei* 0920, stabilized the intestinal microbial system and showed beneficial effects on the caecum microbiota activity of rats (Klewicka et al. 2009). Beet kvass decreases the amount of *Candida*-type yeasts, which cause inflammation, gas, and abdominal pain. The microorganism *Lactobacillus plantarum* is responsible for this effect of kvass. *L. plantarum* can also inhibit the pathogenic bacteria and help to maintain the intestinal microbial balance. Beet kvass when consumed before meals or with meals helps in digestion due to its active enzyme content (Dearie 2016).

In some manuscripts, the term "betanin" was used for standardized *Beta vulgaris* root extract. Kapadia et al. (2003) reported that the standardized beetroot extract (betanin) inhibited *N*-nitrosodiethylamine (30 mg/kg) and phenobarbital-induced liver cancer. The extract also showed chemopreventive effect against 7,12-dimethylbenz(a)anthracene-UV-B-induced splenomegaly with oral administration at the 0.0025% dose. In addition, standardized beetroot extract administered topically at the same dose inhibited (±)-(*E*)-4-methyl-2-[(*E*)-hydroxyamino]-5-nitro-6-methoxy-3-hexanamide and 12-*O*-tetradecanoylphorbol-13-acetate-induced skin carcinogenesis in mice (Kapadia et al. 2003).

In the study conducted by Klewicka (2010), the antimutagenic activity of raw and fermented beetroot juice was investigated. The beetroot juice was fermented with *L. paracasei* (0916, 0920, and 0923) and *L. brevis* 0944. The Ames test was conducted for the determination of antimutagenic activity, in which *Salmonella typhimurium* designated as TA98 and TA100 was used. As a result, the beetroot juice fermented by the *Lactobacillus sp.* 0916, 0920, 0923, and 0944 prohibited *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced mutation as in fresh beetroot juice. It is thought that the antimutagenic activity of the kvass is related to its betalain content and *Lactobacillus* fermentation (Klewicka 2010).

It is demonstrated that the antimutagenic activity of fermented beetroot juice may vary with storage time. It was determined that the antimutagenic activity of fermented beetroot juice did not change for 30 days. After 90 and 180 days, antimutagenic activity decreased drastically by 32% and 41%, respectively, for *Salmonella* TA 98, and by 41% and 22%, respectively, for *Salmonella* TA 100 as compared to the initial level (Klewicka and Czyżowska 2011).

Klewicka et al. (2015) examined the effects of beetroot juice fermented with *L. brevis* 0944 and *L. paracasei* 0920 on gut microbiota, metabolic parameters, and antioxidant potency. For this purpose, *N*-nitroso-*N*-methylurea (NMU)-induced toxicity model was used in experimental animals, because NMU is a mutagen, carcinogen and teratogen which induce aberrant oxidative and biochemical processes and disadvantageous changes in metabolic activity of the gut epithelium. In the study, male Wistar rats were divided into four groups of eight animals in each. The first group fed on basal diet only, while the second group fed on fermented beetroot juice. NMU was administered to the third group in order to evaluate the changes in the gut microbiota, biochemical, and oxidative parameters. The fourth group received fermented beetroot juice with NMU. The fermented beetroot juice decreased the level of cecal glycolytic enzymes such as β-glucuronidase, which was increased by the treatment of NMU. It was observed that the concentration of ammonia in the cecum of rats that were fed fermented beetroot juice was decreased. In animals treated with fermented beetroot juice and NMU, blood serum antioxidant levels increased by 69% compared to animals treated with NMU.

Klewicka et al. (2012) performed a study in order to determine preventive activity of beetroot juice fermented with *L. brevis* 0944 and *L. paracasei* 0920 on aberrant crypt foci in the intestine. In the study, carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine for formation of aberrant crypt foci in rat colon was administered to Wistar male rats by gastric gavage. The average aberrant crypt foci number was decreased by 55% with treatment of fermented beetroot juice. Also, genotoxic evaluation was made in fecal waters of experimental animals that were fed beetroot juice in Caco-2 cell lines by using Comet assay. The results showed that DNA content in the comet tail in the control group significantly decreased compared to that of animals consuming the fermented beetroot juice. Additionally, the fermented beetroot juice increased count of *Lactobacillus*/*Enterococcus* and *Bacteroides–Prevotella* group bacteria in the colonic epithelial tissue.

Vaithilingam et al. (2016) investigated the cytotoxic activity of beetroot juices fermented with *L. acidophilus* NCIM 2903 and *L. plantarum* VITES07 (HF571129) for 24 and 48 hours in human liver cancer cells (HepG2). As a result, the 48-hour fermented beetroot juice exhibited 64% cytotoxic activity, while the reference anticancer drug doxorubicin exhibited 86% activity.

3.5 Storage and Shelf Life

Klewicka and Czyżowska (2011) conducted research to determine the stability and shelf life of beetroot juice fermented with *Lactobacillus brevis* 0944 and *Lactobacillus paracasei* 0920. Fermented beetroot juice was stored for 180 days at 4°C. The changes in the content of betaine, betanidine, and isobetanin were observed during the storage period. Betanin, betanidin, and isobetanin content decreased by 17%, 17%, and 4%, respectively, while the isobetanidin content did not change and the neobetanidin content increased by 54% in the first 7 days. From 7th to 30th day of storage, the content of betalains did not change dramatically. 90 and 180 days after fermentation, the level of betalain decreased drastically by 68% and 75%, respectively, as compared to the initial level. The initial level of *Lactobacillus* sp. was 9.11 $\log_{10}$ cfu/mL in the fermented beetroot juice. 30 and 180 days after fermentation, the colony count of bacteria decreased to 8.15 and 6.80 $\log_{10}$ cfu/mL, respectively. As a result of the study, the optimum shelf life of fermented beetroot juice was determined as 30 days.

4 Conclusion

Kvass made from rye bread, black raspberry, mint, sea-buckthorn, and several vegetables such as beetroot, is a traditional fermented beverage consumed in Russia and Eastern European countries. Kvass produced using the fermentation technique is one of the best sources of probiotics. Furthermore, this fermented beverage is also rich in minerals, amino acids, and vitamins, which are beneficial for human health. Hitherto, scientific studies have been conducted on antimicrobial, antioxidant, antiinflammatory, and anticancer effects of kvass. The average shelf life of kvass depends on factors such as storage temperature and its alcohol content. Over the last 30 years, changes in eating habits and industrialization have led to an increase in nutritional health problems such as obesity, constipation, and dyspepsia. Therefore, it should be recommended that the fermented food products, which are probiotic sources, are frequently consumed.

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18

Kefir and Kombucha Beverages: New Substrates and Nutritional Characteristics

Rafael Resende Maldonado
Food Department, Technical College of Campinas (COTUCA), University of Campinas,
R. Jorge Figueiredo Corrêa, Campinas, São Paulo, Brazil

Elizama Aguiar-Oliveira
Exact Sciences and Technology Department, Santa Cruz State University, R. Jorge Amado,
Ilhéus, Brazil

Eliana Setsuko Kamimura and Mônica Roberta Mazalli
Food Engineering Department, Faculty of Animal Science and Food Engineering,
University of São Paulo, campus Pirassununga, Av. Duque de Caxias Norte,
Campus da USP, Pirassununga, São Paulo, Brazil

1 Kefir and Kombucha: Initial Concepts

Fermentation processes are used as food preservation techniques used by humans for centuries, for the extension of shelf life of different products. The most common forms of fermentation are lactic, alcoholic, and acetic fermentations. The lactic fermentation is commonly used to expand the shelf life and confer new characteristics to different types of milks, and also to prepare them for preservation. The alcoholic fermentation is applied to convert sugary extracts into wines, beers, and other fermented beverages, which can then be distilled originating various beverages of high-alcohol content. The acetic fermentation is mainly used for the production of vinegar, used as a condiment and food additive.

There are numerous microorganisms (isolated species or associations between different species) that can serve as a starter culture for fermentation processes. Kefir and kombucha are two examples of symbiotic associations between microorganisms that are utilized to produce beverages from different substrates. Both are composed mainly of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts (Y), varying on the basis of the species present, the type of culture, and the preferred substrates of action.

Kefir grains are formed by a matrix of polysaccharides in which the microorganisms (LAB, AAB, and Y) are enveloped. The cultivation of the grains can be done both in milk and sugary aqueous solution. The resulting beverage has an acidic taste, a creamy consistency, and a slightly carbonated appearance. The origin of the kefir beverages is attributed to the mountainous regions of the Caucasus and they are widely consumed in Eastern Europe, South-east Asia, and Russia. In recent times, the consumption of kefir beverages has expanded to other regions of the world and the number of studies about kefir has increased significantly due to the identification of various probiotic and functional characteristics that these beverages possess (Fiorda et al. 2017; Gul et al. 2017, 2018).

Milk kefir cultures are formed by grain-shaped clusters that resemble the pieces of coral or cauliflower. They contain a complex mixture of microorganisms—bacteria of various species such as *lactobacilli*, *lactococci*, *leuconostocs*, and *acetobacteria* and yeasts, both lactose-fermenting and nonlactose fermenting. The products obtained from the fermentation of kefir are similar to yoghurt. However, they contain a varied range of substances, such as lactic and acetic acids, carbonic gas, ethanol, and aromatic compounds. From the sensory point of view, the beverages are mildly fizzy, sour, and refreshing. Probiotic characteristics have been usually attributed to kefir beverages due to the numerous types of beneficial microorganisms present in their composition (Otles and Cagindi 2003).

Water kefir grains (or sugar kefir grains) can be cultivated in mixtures of water, dried fruit, and/or sugar, and the fermentation is usually done at room temperature under anaerobic conditions for 2–4 days. The resulting beverage is slightly sweet, acidic, alcoholic, and fizzy, with a yellowish color and fruity aroma. The grains are formed by dextran exopolysaccharides (EPS) and they are translucent, of fragile structure, insoluble in water, and formed by different species of microorganisms (Laureys et al. 2018).

Kombucha is also a symbiotic culture formed by LAB, AAB, and Y. However, the most common substrate for its growth is black tea. During fermentation, kombucha cultures usually form a floating biofilm on the surface of the substrate. This biofilm increases in size during the fermentation period, forming plates, which can be used as inoculum for the propagation of the culture. Kombucha produces different types of acids, which are responsible for its characteristic taste. The kombucha fermented beverage originated in India and China, where it is widely consumed (Villarreal-Soto et al. 2018; Adewumi 2019).

2 Kefir and Kombucha: New Substrates and Production of Beverages

2.1.1 Kefir Beverages

Kefir fermented beverages are traditionally produced from the fermentation of cow milk (using milk kefir grains) or sugary aqueous solution (using water kefir grains). However, other substrates have also been studied for the production of beverages with different flavors, greater nutritional value, and the development of vegan formulations. Other types of milk (goat, buffalo, sheep, camel, and so on), vegetal extracts (soy, nuts, coconut, oat, rice, pumpkin, yam, etc.), fruit juice, and fruit pulp are new substrates (dairy or nondairy) that have been assessed for the production of kefir beverages in function of the variations in the nutritional composition (lactose content, cholesterol, bioactive compounds, among others), flavors and aromas, aiming to reach the most varied types of consumers (Cui et al. 2013; Sabokbar et al. 2015; Nogueira et al. 2016; Randazzo et al. 2016; Wang et al. 2017; Da Costa et al. 2018; Da Graça et al. 2018; Koh et al. 2018; Yépez et al. 2019).

In the production of milk-based kefir beverages, those produced with whole milk have better sensory and physicochemical characteristics in general. On the other hand, it is well known that the excessive consumption of saturated fats and cholesterol is harmful for the health. Another point to be considered is that many consumers are intolerant to lactose and/or allergic to milk proteins. Therefore, several formulations using skimmed milk, low fat content, fat substitutes, and/or vegetal extracts have been assessed in order to meet these diverse nutritional demands (Nielsen et al. 2014).

Vegetal extracts have been a promising alternative in the development of nondairy fermented beverages, especially due to the absence of lactose and cholesterol (present in milk). There are still further advantages in using vegetal extracts, such as the presence of anticarcinogenic substances and polyunsaturated fatty acids (PUFA) (soybean); reduction of hypertensive effect, concentration of LDL-cholesterol, blood glucose and insulin (oat); presence of proteins, amino acids, vitamins (mainly vitamin C) and minerals (cocoa); high nutritional value and high sensory acceptance (walnut); and high phenolic concentration (peanut) (Liu and Lin 2000; Puerari et al. 2012; Cui et al. 2013; Bensmira and Jiang 2015; Dinkçi et al. 2015).

Fruits are another group of substrates that have demonstrated great potential for fermentations with kefir, especially with water kefir. The chemical composition and the sensory quality vary greatly depending on the raw material used. However, in general, the fermented products obtained have a rich chemical composition, containing different types of sugars, organic acids, alcohols, and esters, resulting in refreshing flavored beverages (due to the presence of ethanol), fruity aroma (due to the presence of esters), slightly thick consistency, and smooth texture (due to the presence of glycerol, polysaccharides, etc.) (Fiorda et al. 2017).

In countries such as Canada, the United States and the United Kingdom, there are a wide variety of commercial kefir-based beverages already available for purchase, for example: traditional kefir, lactose-free, low and nonfat, organic, Greek style, vegan (oat, coconut, etc.), flavored (cinnamon, citrus), low calorie, and also derived products such as cheese and frozen kefir (Prado et al. 2015).

The most utilized conditions of fermentation for the production of kefir beverages are at temperatures close to room temperature (20–30°C) and fermentation time of approximately 24 hours. It is possible to verify that the increase in temperature causes a decrease in the fermentation time, whilst the use of substrates which differ from the milk very much can prolong the fermentation process. The inoculation forms vary depending on the usage of grains (usually added at the rate of 5% w/w) or starter cultures (consisting of isolated and concentrated microorganisms from a kefir culture and used at much lower concentrations). The physicochemical, microbiological, and sensory characteristics vary greatly as well, depending on the type of substrate used.

Table 18.1 presents a brief overview of recent studies (2015–2019) for the production of kefir fermented beverages, the substrates used, and the main characteristics of the fermented products obtained. This review is mainly focused on new substrates that have been applied for the production of kefir beverages.

2.1.2 Kombucha Beverages

The beverage fermented by kombucha is obtained mainly from the fermentation of black tea (Camellia sinensis) by a microbial consortium composed of several species, especially bacteria and yeasts. Based on different studies, Jayabalan et al. (2014) indicate that the beverages are rich in different organic acids, such as acetic, gluconic, glucuronic, citric, lactic, malic, tartaric, malonic, oxalic, and succinic. In addition, they may contain fermented sugars (sucrose, glucose, and fructose), vitamins C and B complex, different types of amino acids, biogenic amines, purines, pigments, lipids, proteins, ethanol, carbon dioxide, polyphenols, minerals, hydrolytic enzymes, and substances with antibiotic activity.

Watawana et al. (2017) studied five different kombucha cultures obtained from local producers in Sri Lanka to ferment black tea (1% w/v) with seven different types of sweeteners (aspartame, bee honey, brown sugar, white sugar, glucose, kitul honey (*Caryota urens*), palmyra jaggery (*Borassus flabellier*), and sucrose. The fermentations were conducted at 24°C for 7 days with inoculum of 3% w/v of freshly grown tea fungus. The authors verified that the different types of sweeteners did not alter the antioxidant activity of the fermented beverages significantly, and that the cultures were able to ferment all the mixtures evaluated.

The usage of sweetened (black or green) tea for the production of kombucha fermented beverages, as well as their therapeutic properties, are already well known and described in the literature. In recent years, the search for the use of kombucha in new substrates as a means to obtain products with different chemical, nutritional, and sensory characteristics in relation to the traditional ones has been observed in the literature (Villarreal-Soto et al. 2018).

Other infusions have been assessed, regarding especially the antioxidant activity. Vitas et al. (2018) applied kombucha to ferment the infusion and subcritical water extract (SWE) from

TABLE 18.1

Different substrates for the production of beverages fermented by kefir in recent researches

Culture	Substrates and fermentation conditions*	Physicochemical characteristics	Reference
Milk and water kefir with or without the addition of *L. plantarum* M5MA1-B2	S: Oat, barley, and maize (12% w/v cereal in distilled water) and milk (control condition). F: 25°C, 48 h; I: 6.25% w/w of rehydrated kefir	Milk kefir: greater fermentability, concentration of lactic acid viscosity; the addition of *L. plantarum* increased the viscosity in the water kefir beverages	Yépez et al. 2019
Milk kefir grains (Samsun, Turkey) and kefir starter culture (Danisco, Poland)	S: Standardized cow milk and buffalo milk (11.5% w/w total solids) F: 24°C, 18 h, until pH ~4.5; I: 5% w/w kefir grains or 0.025 g/L of kefir starter culture	Buffalo milk resulted in a fermented beverage with greater EPS production, firmness, viscosity, consistency index, sensory evaluation, and color	Gul et al. 2018
Milk kefir grains (Brazil)	S: Cupuassu (0–100% w/w) + whole cow milk F: 25°C, 24 h I: 5% w/w kefir grains	The increase in the cupuassu concentration increases the cellular growth of the grains and the soluble solids, acidity, and ashes in the beverages	Da Graça et al. 2018
Milk kefir grains (Samsun, Turkey) and kefir starter culture (Danisco, Poland)	S: Cow milk and sheep milk; F: 25°C, until the pH is between 4.6 and 4.7; I: 5% w/v kefir grains and 2% v/v of kefir starter culture activated in pasteurized milk, three times at 25°C for 18 h	The beverages with kefir starter culture presented quicker acidification (4–12 h) and the beverages containing sheep milk and kefir grains presented higher antioxidant capacity	Yilmaz-Ersan et al. 2018
Water kefir grains (Ghent, Belgium)	S: water, sugar, dried fruits (fig, apricot, or grapes), fresh figs, peptone extract and/or yeast extract F: 21°C, 3 days, different aeration conditions I: 22.7% w/w of kefir grains	The increase in aeration favored the production of acetic acid. The use of dried apricots resulted in a higher pH and growth of the grains, whereas the raisins had the opposite effect	Laureys et al. 2018
Water kefir grains (Brazil)	S: Cow milk and water-soluble soy extract F: 37°C, 19 h I: 7.7% w/w of kefir grains	The final acidity varied from 0.600 to 0.738 g of lactic acid/100 g; SS ranged between 5.67 and 6.40 °Brix and the replacement of cow's milk by water-soluble soy extract did not affect the growth of LAB and Y	Norberto et al. 2018
Water kefir grains (Brasília, Brazil)	S: mixtures containing yam extract, sesame, and beans with or without the addition of brown sugar F: 22°C, 24 h I: 5.0% w/w of kefir grains	Supplementation of the substrates with beans extract accelerated the fermentation. The formulation with 50% of beans extract was the best substrate and the beverage was the richest in proteins	Da Costa et al. 2018

(*Continued*)

TABLE 18.1 (Cont.)

Culture	Substrates and fermentation conditions*	Physicochemical characteristics	Reference
Water kefir grains	S: pumpkin purée and brown sugar F: 32° C, 24 h I: 5.0% w/w of kefir grains	The optimized beverage was obtained with 22.28% of pumpkin purée and 9.07% w/w brown sugar, which resulted in 16.39 °Brix, 50.09.10^{-3} Pa.s, pH 4.56, and sensory acceptance of 91.4%	Koh et al. 2018
Freeze-dried kefir microbial mixture (USA) contend LAB, *Candida kefir*e *Saccharomyces unisporus* spp.	S: isolated tomato seed protein, sucrose, and ascorbic acid F: 37°C, 24 h I: 10^9 UFC/mL	The optimized condition for the production of beverages was obtained with 0.22 g/L kefir grains, 6.75 g/L sucrose, and 196 × 10^{-3} g/L ascorbic acid. There was a significant decrease in the protein concentration and increase in the antioxidant activity in function of the production of bioactive peptides	Mechmeche et al. 2018
Kefir starter culture with or without the addition of *Bifidobacterium animalis* subsp. *lactis* BI-400 and *B. longum* BI-5 (Danisco)	S: 15% w/v of water-soluble soybean extract + 85% w/v of cow milk F: 25°C, until pH is 4.6 I: 0.025 g/L kefir culture; 0.010 g/L *B. animalis,* and 0.002 g/L *B. longum*	The formulation with soybean extract and *B. longum* presented greater functionality, more pleasant aromas and a smaller quantity of unpleasant aromas	Karaçalı et al. 2018
Kefir Mild 01 starter culture (Danisco, China)	S: Goat milk + polymerized whey protein (PWP) and pectin F: 28°C, 13 h I: 0.01% w/v	The best formulation (with 0.3% w/v of PWP and 0.2% w/v of pectin) presented a low rate of syneresis (5.44%), good viscosity (951.86 × 10^{-3} Pa.s) and good hardness (112.57 g), similar to that fermented with cow. The average notes for aroma and flavor were 88.2% and 74.4%, respectively	Wang et al. 2017
Kefir starter culture with different strains of *Lactobacillus* (Quebec, Canada)	S: Cow milk supplemented with three fava bean (whole, cotyledon, and hull), chickpea flour (1.5 and 3.0% w/w), chickpea mucilage, inulin (20% w/w) F: Overnight at room temperature I: Not specified	Pulse supplements were more efficient than inulin in maintaining the stability of the kefir beverages during storage	Saadi et al. 2017
Kefir starter culture (Danisco, Barcelona, Spain)	S: Skimmed goat milk powder (SMP) and different types of inulin (native, short-chain, and long-chain) at the concentration of 4% w/w F: 23°C, 24 h I: 0.01% w/w	The final pH values ranged from 4.42 to 4.44, and the apparent viscosity from 87.9 to 100.8 × 10^{-3} Pa.s when a shear rate of 20/s was applied. There was no significant difference in the results related to the use of different types of inulins	Simsek et al. 2017

(*Continued*)

TABLE 18.1 (Cont.)

Culture	Substrates and fermentation conditions*	Physicochemical characteristics	Reference
Milk kefir grains (Federal University of Lavras, Brazil)	S: apple pulp (16.5% w/w of soluble solids) F: 28°C, 5 days with agitation of 150 rpm in the acetic fermentation stage I: 10 or 20% w/w of kefir grains	Vinegar with 4.1% w/v acetic acid was obtained. No significant differences between the vinegars produced and the commercial vinegar were observed in the sensory evaluation	Viana et al. 2017
Kefir starter culture (Tianmei Biotechnology Co. Ltd, China)	S: Skimmed cow milk with soluble solids adjusted to 14% w/w with skimmed cow milk powder and isomalto oligosaccharide (IMO) from 1 to 3% w/w F: 30°C, 16 h I: 0.01% w/w	The use of IMO diminished the separation of whey from the fermented milk and improved the hardness and firmness in relation to the control condition using whole milk. However, the sensory acceptance decreased with the increase of IMO in the formulation	Mei et al. 2017
Milk kefir grains (Tibet) and water kefir (Mexico)	S: Soybean hydrolyzed extract, cow milk, bovine colostrum (for milk kefir), and honey (for water kefir) F: 30°C, 24 h I: 5%w/v of kefir grains	The fermented beverage obtained with honey presented better results (higher antioxidant activity, concentration of lactic acid, yeast population, protective effect against DNA damage, and sensory quality)	Fiorda et al. 2017
Water kefir starter culture (BioNova, Italy)	S: six vegetal juices (carrot, fennel, melon, onion, tomato, and strawberry) F: 25°C, 48 h I: 4% w/v of inoculum from the culture of 0.125 g of the starter culture were utilized in the juices for 25°C, 72 h	All the substrates evaluated were efficient for the growth of the culture. The fermented melon beverage presented the highest final concentration of microorganisms; strawberry, onion, and melon the highest ester concentration, whilst fennel and tomato had highest amounts of terpenes. The strawberry, onion and tomato fermented beverages presented the greatest retention of antioxidant activity and the carrot beverage was the most accepted sensorially	Corona et al. 2016
Water kefir starter culture (BioNova, Italy)	S: s fruit juices (apple, quince, grape, kiwifruit, prickly pear, and pomegranate) F: 25°C, 48 h I: 4% w/v of inoculum from the culture of 0.125 g of the starter culture were utilized in the juices for 25°C, 72 h	The kefir culture was able to ferment all the juices. The highest concentrations of LAB and Y were obtained with the prickly pear; the greatest antioxidant activity with kiwi and pomegranate; and the best sensory acceptance with apple and grape	Randazzo et al. 2016
Milk kefir grains (Greece)	S: Pomegranate and orange	The combined substrates of pomegranate with orange	Kazakos et al. 2016

(*Continued*)

TABLE 18.1 (Cont.)

Culture	Substrates and fermentation conditions*	Physicochemical characteristics	Reference
	F: 30°C, until the ethanol concentration reaches 0.5 to 1.0% v/v I: 8.0 g/L kefir grains (on a dry base)	enhanced the growth and survival of the LAB, and all the formulations obtained high sensory acceptance (>88%)	
Milk kefir and water kefir adapted for milk (Brazil)	S: Milk and aqua mixtures —with aqua concentrations varying between 0 and 90% w/w F: 25°C, 24 h I: 5% w/w of kefir grains	The water kefir culture adapted (with the 30% w/w aqua in the formulation) presented the best sensory acceptance due to the lower acidification during fermentation	Nogueira et al. 2016
Milk kefir grains (Samsun, Turkey) and kefir starter culture (Danisco, Poland)	S: Standardized cow milk and buffalo milk (11.7% w/w total solids, 2.7% w/w fat, and pH 6.68) F: 24°C, 18 h I: 0.025 g/L kefir starter culture	The fermented buffalo milk presented higher concentration of ethanol, microbial population, and sensory acceptance than the fermented cow milk	Gul et al. 2015
Milk kefir grains (Turkey)	S: Goat milk (from two regions of Turkey obtained by intensive and extensive feeding of animals) and cow milk (obtained by intensive feeding) F: 25°C, 20 h I: 2% w/w of kefir grains	The fermented goat milk showed higher microbial population and a high concentration of phenolic compounds (726 to 1359 mg gallic acid per liter)	Satir and Guzel-Seydim 2015
Kefir starter culture	S: Goat and sheep milk with high concentration of PUFA induced by the diet of the animals F: 22°C, until pH is 4.6 I: 7.5 g/100 L	The higher PUFA content increased the fermentation time and did not interfere with the production of ethanol. For the fermented sheep milk, the increased PUFA content reduced the production of 2,3-butadione, the animal odor and increased the creamy aroma. On the other hand, for the goat milk, there was an increase in the lactic acid concentration and a decrease in whey aroma	Cais-Sokolińska et al. 2015
Kefir starter culture (Silver Bay, USA)	S: Mixture of reconstituted skimmed milk (12% w/w) and peanut extract in a 7:3 ratio F: 28°C, 25 h I: 3% v/v of the inoculum prepared with 0.2% kefir starter culture fermented in skimmed milk (12% w/w) at 22°C, 24 h. Control condition: substrate acidified with lactic acid until pH is 4.6	The fermented product presented a higher concentration of phenolic solubles than the control. The main phenolic groups encountered in the fermentation were the phenolic acids and flavonoids	Bensmira and Jiang 2015

(*Continued*)

TABLE 18.1 (Cont.)

Culture	Substrates and fermentation conditions*	Physicochemical characteristics	Reference
Milk kefir grains (Iran)	S: Apple and whey F: 20–30°C, 48 h I: 2–8% w/w of kefir grains	The best condition for the production of the beverage was obtained with 3.73% w/w of kefir grains and temperature at 24.3°C, which resulted in 71% of sensory acceptance. The increase in the concentration of inoculum increased the acidity, production of kefiran, microbial population, and viscosity	Sabokbar et al. 2015

* S = substrates; F = fermentation conditions (temperature and time); I = inoculum condition.

yarrow flower (*Achillea millefolium*) from Siberia. The fermentations were conducted for 7 days at 25°C with 10% v/v inoculum and 70 g/L sucrose. The results indicated that the fermented infusion presented a higher concentration of oxalic acid and higher antioxidant activity than fermented SWE. The beverage obtained with SWE, in turn, presented a greater antimicrobial activity and greater sensory acceptance than the one obtained with the infusion. Furthermore, there was a reduction in the concentration of flavonoids and an increase in the concentration of vitamin C in both fermented beverages in comparison to the substrates. Vázquez-Cabral et al. (2017) evaluated the fermentation of four oak species (*Quercus* spp.) using 10% w/v of starter culture from Mexico to prepare the inoculum. The inoculum was added to the fermentation medium in the proportion of 2.5% w/v and the fermentation was conducted for 7 days at 25°C. The results obtained for the fermented beverages showed that they were able to reduce significantly the levels of proinflammatory cytokines, the production of NO, and oxidative stress in *in vitro* tests.

Milk, which is traditionally utilized for fermentation with lactic bacteria or kefir, has also been the subject of studies for fermentation with kombucha. Kanurić et al. (2018), for example, applied kombucha for fermentation in milk at different pH values (4.6–5.8) and temperatures (37–42°C) and verified that the metabolism of lactose is divided into two stages: an initial retention of the concentration of lactose and then a decrease in the concentration, from 5 to 8 hours of fermentation, depending on the temperature. Vukic et al. (2014) evaluated kombucha starter cultures in different phases of the milk fermentation and the results showed that, in the conditions of initial pH 5.4 and culture temperature of 42°C, there was a similarity of the gelation process compared to fermented milks with probiotic cultures. The analysis of the protein profile showed that the kombucha fermented milks presented better stability of the α and β fractions at certain stages of the fermentation. In another study, Vitas et al. (2013) also verified the possibility of milk fermentation by two cultures of kombucha, one previously cultivated in stinging nettle and the other in winter savoury. Both inocula were able to ferment the milk and increase its antioxidant activity, being the best conditions for cultivation at 41 and 43°C and with a concentration of 2.8% w/w of fat in the milk.

Other substrates have also been the object of study for fermentation by kombucha in recent years. Zubaidah et al. (2018) evaluated the fermentation of sweetened juice of five varieties of snake fruit. The Salak Suwaru variety presented the most acceptable properties: 7.54% w/w total sugar, 11.3% w/w soluble solids, 1.65% w/w acidity, pH 3.15, and sensory acceptance of 78% for color, 74% for flavor, and 76% for aroma. Acetic acid was the main organic acid produced during fermentation, and the beverages presented antimicrobial activity against both Gram-positive and Gram-negative bacteria. There was also an increase in the concentration of phenolics, tannins, and flavonoids throughout the fermentation.

Ayed et al. (2017) and Ayed and Hamdi (2015) evaluated, respectively, red grape juice and prickly pear juice as substrates for fermentation of a kombucha culture from Russia. The fermentations were conducted at 30°C for 12 days in a dark environment using 3% w/w inoculum of cellulose film and 10% w/v of the beverage previously fermented by kombucha. The ideal fermentation time verified in both studies was of 6 days, which resulted in reduction of pH, increase of antioxidant activity, and better sensory acceptance.

Watawana et al. (2016), in turn, worked with coconut water from fresh king coconuts of 7 months of maturity and five kombucha cultures of domestic producers from Sri Lanka. After the fermentation, the beverages presented increased antioxidant activity and yellow color, and reduced pH in comparison to nonfermented coconut water. In another study, Watawana et al. (2015) applied the same conditions of the previous study for the fermentation of coffee-based beverages. Three types of coffee were used: fine ground coffee (FGC), coarse ground coffee (CGC), and instant coffee (IC). There was a significant reduction of the pH from 5.0 to 4.1 (with FGC); an increase in the concentration of caffeic acid (with FGC) and chlorogenic acid (with FCG and CGC) after 7 days of fermentation at 24°C with inoculum of 3% w/v) of the freshly grown tea fungus.

Comparatively, studies on fermentation with kombucha appear in a much smaller number than those conducted with kefir. However, the search for alternative substrates for this type of culture has grown, focusing on obtaining beverages with high antioxidant power and high antimicrobial activity.

3 Nutritional Aspects of Beverages Fermented by Kefir and Kombucha

3.1.1 Kefir Beverages

The market for functional beverages is a growing sector of the food industry, since consumers are increasingly concerned about health issues and, therefore, looking for foods that contribute to the prevention of diseases. Fermentative processes are ancestral means of preserving food, which enhance its nutritional characteristics as well. The fermentation of milks, grains, and other substrates to obtain health-promoting beverages is a well-known strategy in several regions of the world (Marsh et al. 2014).

In this context, several studies have investigated the benefits of beverages fermented by kefir. Such beverages have a positive effect on the gastrointestinal tract. The regular consumption of kefir-based beverages has often been associated with the improvement of digestion and tolerance to lactose. In addition, fermented milks may contain components that are not naturally present in milk, such as EPS, kefiran, folates, vitamin B12, and riboflavin, all of them having a beneficial effect on the health. Furthermore, fermented dairy products typically present β-galactosidase activity, which reduces the concentration of lactose, making these products more acceptable than milk *in natura* for the lactose-intolerant people (Hugenholtz 2013; Corgneau et al. 2017; Rosa et al. 2017; Turkmen 2017).

A further relevant aspect of the fermented dairy products is the presence of bioactive peptides, originated from the hydrolysis of proteins by the action of kefir microorganisms during the fermentation. Such peptides have been associated with various beneficial effects on the digestive, endocrine, cardiovascular, and nervous systems (Martinez-Villaluenga et al. 2017).

The regular consumption of products fermented by kefir has also been associated with improvements in the immune system due to the high concentration of probiotics contained in the kefir grains, which have the ability to modulate immunological mechanisms (Davras et al. 2018; Pacini and Ruggiero 2017; Santiago-López et al. 2015). Studies in mice with induced bowel inflammation showed that consumption of kefir caused relief in inflammatory bowel disease, mediated antiinflammatory effects, and created a protection against colitis (Fábrega et al. 2017; Seo et al. 2018).

A major contemporary concern in terms of chronic degenerative diseases is cancer, one of the biggest problems in the world regarding human health. This scenario has caused the demand for forms of prevention to increase, especially those related to the consumption of functional foods. Kefir beverages, due to their powerful probiotic potential, have been related to anticancer properties. The antimutagenic action, the antitumor properties, and the presence of LAB in kefir beverages play an important role in the prevention of cancer. Numerous studies have indicated beneficial effects of kefir consumption on different types of cancer, such as gastric, colorectal, breast, lung, and malignant T lymphocytes (Rizk, Maalouf and Baydoun 2009; Maalouf et al. 2011; Kuo et al. 2013; Amiot 2014; Aragon et al. 2015; Ghoneum and Felo 2015; Giovanni et al. 2015; Tomasello et al. 2015; Zamberi et al. 2016; Dos Reis et al. 2017; El-Din et al. 2017; Sharifi et al. 2017; Ma'mon et al. 2018; Sharma et al. 2018).

The American Heart Association (AHA), the Centres for Disease Control and Prevention and the National Institutes of Health estimated that 92.1 million American adults have at least one type of cardiovascular disease. Projections for 2020 indicate that 43.9% of this population will develop some type of cardiovascular disease. The main risk factors that contribute to cardiovascular diseases include simples habits (smoking, physical activity, and diet) and clinical factors (control of cholesterol levels, blood pressure, and blood glucose) (Mozaffarian et al. 2016).

In vivo studies utilizing mice with obesity induced by a high-fat diet demonstrate that the consumption of kefir has potential to improve the metabolic dysfunctions associated with obesity. In addition, kefir may also decrease the amount of hepatic triacylglycerols due to the reduction of the fatty acid synthase expression, since it acts on the gene involved in the liver lipogenesis (Bourrie et al. 2018). In another study, kefir peptides prevented hyperlipidemia and obesity by modulating the metabolism of lipids (Tung et al. 2018). Brasil et al. (2018), in turn, verified the reduction of blood pressure; cardiac and renal hypertrophy; the inhibition of angiostensin-converting enzyme (ACE); and the reduction of the inflammatory state in hypertensive mice due to the consumption of kefir.

The high antioxidant activity of kefir beverages indicates their potential as natural antioxidants. The supplementation with probiotics increases the antioxidant resistance and the amount of antioxidant enzymes in the body. The accumulation of EPS during the fermentation by kefir has been assigned as a factor of the antioxidant activity increase *in vitro* (Chen et al. 2015; Luang-In and Deeseenthum 2016; Heshmati et al. 2018).

A further property that has been attributed to kefir is the ability to accelerate cicatrization by inducing the migration of fibroblast cells (action found *in vitro*) and modulating the expression of il-1β, tgf-ß1, and bfgf genes *in vivo* (Oryan et al. 2018).

3.1.2 Kombucha Beverages

Fermented beverage from kombucha consists of sweetened fermented tea. It is very popular in China, where it originates from. However, it has also become widely appreciated throughout the world in recent years (Marsh et al. 2014). The kombucha contains polyphenols; fibers; ethanol; minerals; vitamins C and B complex; gluconic, glucuronic, and lactic acids; amino acids; antibiotics; and a variety of micronutrients produced during fermentation (Jayabalan et al. 2014; Baschali et al. 2017; Özdemir and Çon, 2017; Kapp and Sumner 2018). The production of kombucha and the formation of its beneficial components are the result of the metabolic activities of the fermentative microflora and the composition of the tea itself, which is rich in antioxidant compounds (Özdemir and Çon 2017).

One of the main metabolites of kombucha is the glucuronic acid, which is a result of the symbiotic relationship between AAB and Y present in the kombucha microflora (Özdemir and Çon 2017). The presence of this acid improves the detoxification of the human body because the acid binds to the molecules of toxins, forming products that are excreted by the kidneys. The beneficial health properties attributed to kombucha have been associated more with its acidic composition than with its microbial interaction with the human intestine (Wang et al. 2014).

Kombucha has been utilized for several purposes, such as the reduction of cholesterol, blood glucose, hypertension, and cancer spread, as well as to enhance the function of the hepatic, renal, and gastrointestinal systems. *In vivo* studies revealed that the administration of kombucha induced beneficial effects of hypercholesterolemia, particularly with regards to hepatic and renal functions (Bellassoued et al. 2015). It even had a protective effect against hepatic damage induced by thioacetamide in mice (Kabiri et al. 2014). There are also reports of the action of the beverage in the protection of the hepatocytes against lipid toxicity by means of the metabolic regulation of lipids; in the reduction of inflammation and fibrosis, which contributed to the restoration of the liver in mice with nonalcoholic steatohepatitis (Hyun et al. 2016; Lee et al. 2018).

The consumption of kombucha prepared from green tea reduced significantly the parameters of glucose and lipids in the blood, LDL-cholesterol, total cholesterol and triglycerides, and increased the HDL-cholesterol (Rasouli et al. 2014; Hosseini et al. 2016). Other studies have shown that, in comparison to black tea, the kombucha was a better inhibitor of α-amylase and lipase activities in plasma and pancreas; a suppressor of the increase of blood glucose besides inducing the lower absorption of LDL-cholesterol and triglycerides and increasing HDL-cholesterol (Aloulou et al. 2012). The kombucha compounds have also been able to inhibit the ACE and, therefore, can be used clinically to treat human hypertension (Elkhtab et al. 2017).

Diabetes is a degenerative disease associated with an increase in oxidative stress, since the auto-oxidation induced by glucose hyperglycemia and the glycation of proteins produce reactive species of oxygen and nitrogen. Kombucha protects against oxidative stress and inflammatory processes, and has proven to be a potent and significant antidiabetic beverage, since it effectively restores the pathophysiological alterations in mice with diabetes induced by alloxan, especially due to the activity of antioxidant enzymes, which were modified in their pancreatic, hepatic, renal, and cardiac tissues (Bhattacharya et al. 2013).

Vázquez-Cabral et al. (2017) used oak leaf infusions instead of black tea as a substrate to prepare kombucha and obtained reduced levels of proinflammatory cytokines (IL-6 and TNF-alpha) and downregulated the production of nitric oxide due to the phytochemical compounds present in the beverage. Most of the beneficial effects of tea are attributed to its antioxidant and free-radical scavenging properties (Hrnjez et al. 2014). In addition, the presence of polyphenols, organic acids, and B-complex vitamins may also have contributed to the antihyperglycemic effect of kombucha in guinea pigs with diabetes induced by streptozotocin (an antibiotic that damages the pancreatic β-cells) (Srihari et al. 2013a). In another study, a beverage developed with snake fruit (*Salak Suwaru cultivar*) fermented by kombucha was effective in the control of hyperglycemia when administered in doses between 5 and 15 mL/kg body weight/day in diabetic animals (Zubaidah et al. 2018).

Studies have also shown the potential of kombucha against cancer. The beverage inhibits angiogenesis by means of changes in the expression of angiogenic stimulators and decreases the survival of prostate cancer cells, which makes it useful for the treatment and prevention of this type of cancer (Srihari et al. 2013b). Furthermore, in a dose-dependent action, kombucha also inhibited significantly the growth of bladder cancer cells (Kim et al. 2016). In view of the above, kefir and kombucha have been designated as functional beverages for consumers who crave for health and wellness.

4 Final Considerations

From the details discussed in this chapter, we may conclude that there is a great interest in both the production of beverages fermented by kefir and kombucha and their nutritional and therapeutic properties. The literature on kefir beverages is much more prolific and in-depth. However, studies on kombucha have become more numerous in recent years. Some characteristics shared by both microbial cultures are their: varied microflora involving LAB, AAB, and Y; ease to achieve the culture conditions (at room temperature and without stirring); fermentative capacity on different types of substrates; and numerous nutritional and therapeutic effects on the human health. With regards to the production of the beverages, innovation in this area is sought, focusing on obtaining new products, improvement of the nutritional quality (e.g., the association between positive characteristics of substrates and microbial cultures), and meeting the demands of specific groups (intolerant, allergic, individuals with chronic diseases, vegans, and so on). With regards to the nutritional and therapeutic aspects, many studies indicate the benefits of kefir and kombucha fermented beverages mainly for improving bowel function and for preventing or minimizing the effects of chronic diseases (such as cancer, diabetes, hypercholesterolemia, and hypertension). The studies on fermented kefir and kombucha are highly significant and promising in the field of fermented and functional foods. Furthermore, there is also a broad consumer market in some countries and a great potential in regions where these beverages are still less well known.

5 Abbreviations

AAB—acetic acid bacteria
EPS—exopolysaccharides
LAB—lactic acid bacteria
PUFA—polyunsaturated fatty acids
Y—yeast

6 Units

% w/w = g/100 g
% w/v = g/100 mL
% v/v = mL/100 mL
°Brix = g sucrose/100 g of beverage

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Beer between Tradition and Innovation

Maurizio Ciani, Laura Canonico and Francesca Comitini
Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

1 Introduction

Beer is one of the most consumed alcoholic beverages in the world and represents an example of the oldest biotechnology with its origins dating back to around 10,000 years ago (Lodolo et al., 2008). Until 2012, the global beer market showed an increase in both production and consumption, stopping in 2013. According to data from the latest Barth Report, world beer production in 2017 was around 1,952 million hl versus 1,950 of the previous year, with a slight increase of 0.1%. However, the global data of consumption and production showed differences at country level (www.beverfood.com/mercato-mondiale-birre-consumi-produzione-fermi-1952-mn hh_zwd_119344/). In 2017, China is confirmed as one of the first brewing countries with 440 million liters of beer produced, the US market is in the second place with a production of 218 million hl. The other two most important beer markets of the American continent are Brazil (140 million hl, in third place in the world ranking) and Mexico (110 million hl, in fourth place in the world rankings). In Europe, the record of production and consumption belongs to Germany with 93 million hl of production, followed by Russia (74 million hl produced). The other European countries that rank among the top 10 breweries in the world are in the UK, Poland, and Spain.

In recent years, new market perspectives are emerging since consumers focused their attention toward beer culture based on a beer characterized by a peculiar aromatic taste but also focused on its nutritional components. For these reasons, brewers payed attention on the selection of hop varieties, malts, yeast and on fermentation management (King and Dickinson, 2003; Vanderhaegen et al., 2003; De Keukeleire et al., 2010; Chen and Xu, 2013; He et al., 2013; Pires et al., 2014; Stewart, 2016).

This chapter will be describe all the steps of brewing process focusing the attention on the recent biotechnology novelties as the new fermentation strategies to enhance flavor and improve complexity of beers.

2 Brewing Process and Raw Materials

The brewing process, can divided in four steps: malting, mashing, fermentation, and downstream.

Malting is a phase where different enzymes are activated to degrade starch, proteins, and other component of cereals. This step is crucial to obtain sugar, amino acids and fatty acids to support the yeasts' growth. To obtain this objective, barley is placed in water at 10–15°C and germinated for 3–7 days. After this period, α-amylase, β-amylase, and protease are activated. The malt is dried under controlled conditions to remove the water and inactivate the undesired enzyme. During the drying phase, the color and flavor are affected by this step.

During the mashing, the malt is mixed with water and the unfermentable sugars and proteins are hydrolyzed by enzyme of the malt to obtain wort fermentable by yeasts (Rehberger et al., 1995; Kunze, 1996; Goldammer, 2000; Boulton & Quain, 2006). The wort composition is related to ingredients the technology process and could affect the fermentative yeast performance (Meilgaard, 1976; Gunkel et al., 2002; Van Nierop et al., 2006). After that, the malt is mixed with water and heated (Montanari et al., 2005). Generally, the mash program starts with a temperature of 45–55 °C to enhance the protease activity that splits proteins into polypeptides, which in turn are degraded by enzymes into peptides and free amino acids. The second step is carried out at a temperature of 58–63 °C, which is the optimal interval of temperature for the action of β-amylases which degrade the starch to give rise to maltose, the most important fermentable sugar present in wort. The third step occurs at 68–73 °C to have maximum activity of the α-amylases which degrade the starch in a random manner, producing mainly dextrins, carbohydrates with a medium/low molecular weight not fermentable from *S. cerevisiae*, but still important because they positively affect the final body of beer. Temperature variations of only 2–3 °C for a few minutes can significantly change the maltose/dextrin ratio. Therefore, the concentration of sugars present in the wort is variable and depends on the type of malt, on the action of α and β-amylases, and on the operating conditions. Indeed, temperature, time duration, and pH affect the enzymatic activity with repercussions on the final characteristics of the beer, that may be 'full-bodied' (body conferred by dextrin) or alcoholic (maltose fermentation) (Montanari et al., 2005). Finally, the temperature is brought to 78 °C to inactivate all the enzymes present.

To obtain the optimal enzyme activity, it is important an adequate pH value. Normally the malt has a pH value around 5.8, but this does not correspond to the optimum activity of the enzymes involved, that corresponding to a pH value of 5.2. The optimal range value of pH and temperature for enzyme activity is reported in Table 19.1.

Once the wort is obtained, a filtration is carried out, which allows the separation of liquid fraction from solid one. The grains generally undergo a drying process and are destined for other uses, such as the production of animal feed. The filtered wort is cooked in special boilers, traditionally made of copper but nowadays in stainless steel, where the temperature is gradually raised until boiling. The boil time, between one and two and a half hours, is essential for the type of beer you want to produce (Manzoni, 2006) because the boiling of the wort leads to:

- Must sterilization (essential for subsequent optimal yeast fermentation);
- Inactivation of enzymes;
- Improvement in the extraction of essential oils and resins from hops;

TABLE 19.1

Value of pH and temperature for enzyme activity

Enzyme	Optimum temperature (°C)	Optimum pH	Function
Fitase	30–52	4.4–5.5	Lower pH of wort
β-glucanase	36–45	4.5–5.0	Degradation of β-glucan
Peptidase	46–57	4.6–5.2	FAN production
Protease	46–57	4.6–5.2	Degradation of proteins
β-amilase	62–65	5.4–5.6	Degradation starch
α-amilase	72–75	5.6–5.8	Degradation dextrins

- Precipitation of insoluble substances (hot trubs) responsible for turbidity, following a reaction between malt polyphenols and hops and malt proteins, thus favoring clarification of the wort;
- Color development;
- Evaporation of unwanted volatile compounds;
- Evaporation of excess water and consequent concentration of wort (Harrison, 2009).

During boil, the hops are added to the beer. In this step, this fundamental ingredient exploits its flavoring, bittering, and bacteriostatic capacities. It can be added by one or more ranges and at different times of boil (Simpson, 1993). Moreover, during boil, the wort increases its color and acquires reducing power thanks to the formation of melanoidins, which use part of the oxygen dissolved in the oxidation processes. Melanoidins, together with the presence of acid substances introduced by the hops, determine a decrease in pH (Manzoni, 2006).

Once the cooking phase is finished, the so-called hot trubs are separated by using centrifuges and whirlpools; at this point the must (at 95 °C) is sent to a heat exchanger for cooling and when the temperature falls below 60 °C, there is a further precipitation, resulting from the degradation of proteins and polyphenols. Although both precipitates exert a negative action on the fermentation, they tend to adhere to the cell wall of the yeasts slowing down their activity. Generally only the hot trubs are eliminated, as the removal of the cold trubs determines an attenuation of the characteristics organoleptic of beer. The temperature is lowered to 10–20 °C, depending on the desired beer: low or high fermentation (Cabras and Martelli, 2004; Manzoni, 2006).

The wort obtained was inoculated with yeasts to start the fermentation process. A good fermentation depends on the characteristics of the yeast used, which must come from pure and selected cultures, must be highly vital and have a good resistance to alcohol. For the optimal growth rate of yeast culture, it is important to monitor certain parameters, such as nutrient intake, correct inoculum, dissolved oxygen and temperature. Moreover, for a high-quality beer, it is important that there is a sort of balance between the absorbed nutrients and the released products (Lodolo et al., 2008).

The fermentation process is divided into two phases: primary fermentation and secondary fermentation (maturation). The duration of the primary fermentation is short (about 7 days) and very vigorous, where almost all sugars are fermented and most of the secondary compounds, associated with the aroma of the beer, are formed. At the end of this phase, most of yeast biomass is collected and separated from the beer that undergoes a secondary, long lasting (from 3 to 5 weeks) and slight fermentation carried out by the yeast cells still present. The latter are responsible for the fermentation of residual sugars, remove unwanted compounds, and refine the taste of beer (Sols et al., 1971). In this phase, the temperature is gradually lowered until it reaches values of 0–2 °C, for the low fermentation and 7–10 °C for the high fermentation. During maturation, there are three fundamental steps that transform the still immature beer (green beer) into mature beer: saturation of carbon dioxide, clarification and refinement of taste and aroma. The production of carbon dioxide derives from the metabolic activity of the yeasts not removed at the end of the primary fermentation. The clarification occurs following the precipitation of residual yeast and tanno-protein flocculi on the bottom of the tank. The refinement of taste and aroma is due to the attenuation of the bitterness of the hops and the harmonization of the aromatic compounds present (Cabras and Martelli, 2004; Manzoni, 2006).

During the fermentation process, temperature and humidity must be kept constant to ensure an adequate yeast growth rate. The pH is another parameter to keep under control, that goes from a pH value of about 5.2–5.3 of the must to a pH value of about 4.1–4.2 at the end of fermentation (slightly more marked decrease in Ale beer). This acidification is important for preserving the final product and inhibiting bacterial growth (Harrison, 2009).

3 Raw Materials

The principal ingredients used in brewing process are water, generally malted barley, hop, and yeast.

3.1 Malt

Barley (*Hordeum vulgare*, *Graminaceae* family) is one of the most important cereals grown in the world, which has an annual production of 170 million tons, of which about 20% is transformed into malt in the first phase of brewing. The qualitative parameters of the barley for the brewing process are the caliber and homogeneity of kernels, the resistance to plant diseases of the cultivation area, the low protein content and the rapid water absorption. The germinative energy, the enzymatic potential, and the extract yield must be also high. Another important parameter is the humidity of the seed that varies from 12 to 20% in the collection but must not exceed 15% in the storage phase (Cabras et al., 2004). Indeed, the best beer is produced with barley that is suitably dried after harvest (contained in humidity equal to 10–12%). Overheating of the cereal during drying can make it unsuitable for the malting process and even a potential germination results in negative influences on the final product (Harrison, 2009).

The composition of barley is as follows: 70–85% of carbohydrates, 10.5–11.5% of proteins, 2–4% of organic substances (phosphates, silicates and potassium salts), 1.5–2% of lipids and 1–2% of other substances. The carbohydrates present are starch, simple sugars (sucrose, glucose and fructose), cellulose, and hemicellulose. Of these the starch, which represents 55–56% of the total barley that, after hydrolysis, turns out to be the main source of carbohydrates used by yeasts during the fermentation process. Cellulose and hemicellulose, that give mechanical resistance and rigidity to the seed, are important in beer process since they facilitate the filtration process.

Only one-third of all the proteins present in barley are found in the finished beer and some of these play a positive role since they are fundamental for the formation of foam, improving grain size, persistence, and compactness. On the other hand, an excess of protein are negative since they cause veiling and clouding. Barley proteins can be divided into four groups: gluteline (30%), prolamine (37%), globulins (15%), and albumins (11%). Prolamines are the main proteins that are degraded by enzymes during the malting and mashing process, giving rise to polypeptides and free amino acids used by yeasts; globulins can solubilize and be responsible for cloudiness; finally, albumins are the most soluble and subject to enzymatic degradation.

Lipids, present in the form of triglycerides, have a negative effect on the quality of beer, in particular on the quality of the foam.

The barley also contains small amounts of vitamins (B1, B2, C, E), important for yeast metabolism, and polyphenolic substances, which if solubilized may be responsible for a sour taste and cloudiness (Cabras and Martelli, 2004).

3.2 Hop

The female inflorescence of hops (*Humulus Lupus* L.) is used in the brewing process (Johnstone, 1997; Behre, 1999). The role of hop in beer biotechnology is related to role of conservation, bitterness, and flavor (Barth and Meier, 2012; Steenackers et al., 2015). During the brewing process, hop is added during the boil of wort and α-acids are isomerized into iso α-acids, responsible for the bitterness of beer. During this step, the main aroma compounds of hops are vaporized (Dresel et al., 2013; Forster et al., 2013; Inui et al., 2013), so the brewers through "dry hopping" added hops in cold wort and some important aroma compounds as well.

3.2.1 Hop Aroma

The hop volatiles and their transformation have an impact on beer flavor. The main compounds that contribute to hoppy beer flavor are linalool, geraniol, β-damascenone, β-citronellol, esters, and organic acids (Retrberg et al., 2008). As reported by a different research, the hoppy beer flavor is also related to the interaction (synergistic and masking effect) with other compounds in beer (Hanke et al., 2009; Praet et al., 2012). The modalities to obtain beer with hop aroma are two: late hopping and dry hopping. Late hopping consists in the addition of hop toward the end of kettle boil or to the whirlpool. Late hopped beers contain only trace levels of monoterpene and sesquiterpene but contain relevant amount humulene epoxides or linalool oxides, hop-derived ketones, and esters. Flavor descriptors used for late hopped beers include spicy, noble, herbal, woody, and fruity (Eyres et al., 2009). Dry hopping is a technique, in which hop is added to primary or secondary fermentation which allows a cold extraction in an aqueous ethanol solution (Eyres et al., 2009; Almaguer et al., 2014).

Esters such as nerol and linalool affect the citrus flavor; linalool, geraniol, β-ionone, and citronellol give a flora and fruity flavors (Marriott, 2001), while herbal flavors can be attributed to oxidized sesquiterpenes (Kowaka et al., 1983). The threshold value of this aroma compounds is related to the medium analyzed and especially to the interaction with other compounds (Schönberger and Kostelecky, 2011).

Another important aspect that affects the flavor is a biotransformation of some hop compounds by yeast (King and Dickinson, 2000; Takoi et al., 2016). Indeed, during fermentation, yeast acts on volatiles and non-volatile precursors to produce many compounds responsible of hoppy beer aroma (Kishimoto et al., 2005; Kollmannsberger et al., 2006; Koslitz et al., 2008; Nielsen, 2009). During the fermentation process, monoterpene alcohols are subjected to various chemical modifications (King and Dickinson, 2000, 2003; Praet et al., 2012; Steyer et al., 2013; Takoi et al., 2016). During fermentation, geraniol is transformed into β-citronellol and linalool, while linalool and nerol isomerize into α-terpineol.

The hop essential oils are made up of myrcene, α-humulene, and β-caryophyllene (monoterpenes and sesquiterpenes) that represent up to 80% of total volatiles of hop depending on hop varieties. Myrcene, α-humulene, and β-caryophyllene are only found in dry hopped beers (Fritsch et al., 2005) since a large quantity is lost for evaporation in the fermentation tank (Kishimoto et al., 2005). However, these compounds are converted in flavor-active aroma during oxidation and wort boiling (Praet et al., 2014, 2015). Myrcene is the principal volatile of most hop varieties and it is related to resin-, pine-, and herb-like and significantly contributes to fresh hop aroma. Its concentration decreases strongly during aerobic hop storage and during the brewing process (Steinhaus and Schieberle, 2000; Nance et al., 2011).

β-damascenone is a norisoprenoid that has been found in hop and beer. This aroma compound is related to a fruity and honey-like aroma and its concentration in beer increases during long-term storage or forced aging (Chevance et al., 2002; Kishimoto et al., 2005; Eyres et al., 2009).

3.2.2 Hop Bitterness

The primary contribution given by the hop is the bitterness. During wort boiling, the alpha-acids are converted into iso alpha acids that are the main contributor to bitterness, but also affect the foam stability and inhibit the growth of *Lactobacillus* spp. (Kunimune and Shellhammer, 2008). Other different compounds are related to bitterness, and, among them, isoHumulones is a primary contributor to the bitter taste of beer. Also, in this case the perception of bitter is related to the synergistic or antagonistic effect with another compound.

Regarding beta-acids, they generally are not linked to bitterness and they are lost in brewing process while polyphenols contribute to bitterness and astringency.

3.2.3 Hop Functional Properties

Recent investigations have noted that the active compounds of hops possess functional properties. Indeed, 20–30% of phenolic compounds in final beer come from hop, while 70–80% from malt. Hops contain different flavonoids and phenolic acids, and recently several investigations also revealed the new hop flavonoid compounds with antioxidant, anticancerogenic, antimicrobial and estrogenic properties of beer (Benitez et al., 1997; Miranda et al., 2000; Prencipe et al., 2014; Mudura and Coldea, 2015). However, many technological and analytical aspects of hopping are not yet fully known.

3.3 Brewing Yeasts

The quality of beer is related to the choice of the raw material, but the selection of yeast plays a relevant role. Indeed, yeast is important for several reasons: (i) the fermentative performance such as fermentation rate, ethanol yield, sugar metabolism, (ii) the capacity to tolerate environmental stress and consequently yeast management; (iii) the influence on final aroma (Vanderhaegen et al., 2003; De Keukeleire et al., 2010; Steensels and Verstrepen, 2014; Stewart, 2016; Capece et al., 2018). Yeast cultures belonging to the genus *Saccharomyces* are mainly used in the brewing process (Lodolo et al., 2008). The increase in the number of breweries and the demand of consumers towards beer with complex sensory characteristics led the brewers to selection and use of autochthonous yeasts, spontaneous fermentation, and the use of non-conventional yeasts (non-*Saccharomyces*) to obtain distinctive beers (Basso et al., 2016; Varela, 2016).

3.3.1 **Saccharomyces** *Starter Yeasts*

Saccharomyces yeast species are the most important yeasts used in brewing process and are divided into two groups: top-fermenting yeasts (ale) and bottom-fermenting yeast (lager).

Top fermenting ale yeasts (*Saccharomyces cerevisiae*) are characterized by fermentation temperature of 18–22 °C with a maximum growth temperature of 37 °C, fermentation period of 4–6 days and the resulting beers characterized by fruity aroma (Polaina, 2002). Ale yeasts are used for different beer styles and actually are widely used for most of craft beers' production, mainly for its reduced fermentation time and low processing cost. Bottom-fermenting lager yeasts (*Saccharomyces pastorianus*) are used at fermentation temperature of 8–14 °C with a maximum growth temperature of 34 °C and the beers obtained with these yeasts undergo a low temperature period of maturation (Petruzzi et al., 2016; Budroni et al., 2018). *S. pastorianus* strains are hybrids of *S. cerevisiae* and *Saccharomyces eubayanus* (Dunn et al., 2008; Nakao et al., 2009; Libkind et al., 2011). *Saccharomyces bayanus* showed a low sequence identity (92.5%) when compared with *S. pastorianus* genome. Actually, lager yeasts are classified as allopolyploid hybrids of *S. cerevisiae* and *S. eubayanus.* For the first time, a strain of *S. eubayanus* with high identity (99.5%) with *S. pastorianus* genome was isolated in Patagonia (Dunn et al., 2008; Nakao et al., 2009; Libkind et al., 2011).

The differences regarding these yeasts are not just related to unlike fermentation temperature but also at different metabolic behavior in fermentation. Indeed, lager yeasts differ from ale yeasts in assimilation of maltose and maltoriose and they are more able to utilize maltotriose in comparison with ale strains (Zheng et al., 1994; Stewart, 2010). Another difference is related to production of metabolite compounds released in beer. Lager yeasts are characterized by a higher production of sulphur dioxide than ale yeasts. This property is related to different sulphur metabolism and the low fermentation temperature used during the production of lager beer (Stewart, 1981; Crumplen et al., 1993). Moreover, lager strains produce more diacetyl and vicinal diketone (VDK) metabolites such as α-acetolactate and diacetyl than Ale strains (Gibson, 2003). *S. pastorianus* can metabolize melibiose due to the presence of enzyme α-galactosidase that hydrolyzes this sugar into glucose and galactose that are used in the metabolic pathway of lager strains (Naumov et al., 1995).

3.3.2 **Brettanomyces** *Yeasts Spontaneous Fermentation*

The use of selected starter cultures is a practice used in different industrial fermentation processes where an efficient and controlled process is fundamental. Nowadays, in brewing and in winemaking, the producers are revisiting the practice to rely on the local microflora to introduce more character, complexity, and/or authenticity in their products through the spontaneous fermentation (Steensels and Verstrepen, 2014).

In brewing, the most famous style of traditional beers obtained without any microbial starter inoculum is Belgian acid beers such as lambic, gueze and Rodenbach and Americ colship ale, an American descendant of Belgian Lambic beer (Verachert et al., 1995; Bokulich et al., 2012). The lambic beer is produced in Payottenland, a valley on the western side of Bruxelles on the Senne river. The production of this typical Belgian style is carried out by uncontrolled fermentation carried out by microorganisms that colonized the breweries that start when the boiled wort, into a 'coolship' exposed to air during overnight, is 'inoculated' by autochthonous yeasts and bacteria. After that, the wort is transferred into oak casks to continue the fermentation and the aging for 1–3 years. During the fermentation process, several yeasts species and bacteria develop. Several studies showed that each brewer is characterized by a specific microbial community. In general, the spontaneous beer fermentation is shared in four steps. The first step of fermentation is characterized by Enterobacteria (*Klebsiella* spp., *Enterobacter* spp., *Escherichia coli*, *Citrobacter*, *Serratia*, and *Pectobacterium* and non-*Saccharomyces* yeasts, primarily *Kluyveromyces spp.*, *Hanseniaspora uvarum*, and *Rhodotorula* in coolship ale) (Bokulich et al., 2012; Petruzzi et al., 2016). During this step, the pH value and glucose content decrease, but several aromatic compounds that characterize this style of beer like 2,3-butanediol; ethyl acetate; higher alcohols; and acetic, lactic, and succinic acids, are produced (Martens et al., 1992). The second step is characterized by *Saccharomyces* yeasts, such as *S. cerevisiae* and *S. bayanus*, lactic acid bacteria, mainly *Pediococcus* spp., and acetic acid bacteria. During this step, there is an increase in acidification during approximately 3–4 months and *S. cerevisiae* strains are able to consume the fermentable sugars. Successively they are replaced by the *Brettanomyces* spp., generally *Brettanomyces bruxellensis* (Petruzzi et al., 2016). *Brettanomyces* spp. are the predominant microorganism during maturation which can remain for several years since they may ferment complex carbohydrates such as maltotetralose and maltopentose (generally not used by *Saccharomyces* spp.). In this step, many aroma compounds that characterize this beer style are produced.

4 Bioflavor in Beer

The beer flavor is related not only to the choice of raw material, but specially to yeasts. During the fermentation process, yeast is able to convert the wort sugar in ethanol, carbon dioxide, and volatile compounds through metabolic activities. The main volatile compounds obtained during fermentation process have an impact on the aroma and taste of beer. The by-products obtained during the fermentation process come from carbohydrates and assimilable nitrogen sources (amino acids, ammonium). The wort-free amino nitrogen (FAN) affects the yeast's metabolism in the production of esters, higher alcohols, and vicinal diketones.

Nowadays, the consumers require beer style with a peculiar flavor. For these reasons, the brewers paid more attention on the use of yeast. Many lines of research focused on the selection of new starter strains isolated from non-brewing environments such as wine, artisanal sourdough, bakery, and apple stillage (Canonico et al., 2014; Del Caro et al., 2015; Rossi et al., 2018). Rossi et al. (2018), testing 12 strains coming from these environments, found that they exhibited a superior fermentative attitude than commercial starter strains and produced appreciable quantity of aroma compounds to obtain beer characterized by a distinct flavor. The use of baker's yeast strains as starter cultures for craft beer production has been evaluated at laboratory and brewery scale and the results showed that these strains could allow the transfer of novel properties to beer (Del Caro et al., 2015). The influence of *S. cerevisiae* coming from different matrix was also evaluated in craft beer re-fermentation, which highlighted significantly higher levels of the volatile compounds that are responsible for fruity and flowery aromas (i.e. isoamyl acetate, ethyl octanoate, ethyl dodecanoate phenyl ethyl acetate, β-phenyl ethanol). Great attention was focused on the genetic improvement of brewer's yeast to optimize the fermentation attitude but also the volatile profile (Saerens et al., 2010; Zheng et al., 2012; Ekberg et al., 2013; Gadalupe-Medina et al., 2013). Another approach to obtain an innovative product is the use of non-conventional yeasts (non-*Saccharomyces*) (Budroni et al., 2017). The impact of non-conventional yeasts used in pure and mixed fermentations with *S. cerevisiae* on the flavor profile of other fermented and distilled spirit beverages, has been revaluated (Comitini et al., 2011; Jolly et al., 2014; Ciani and Comitini, 2015; Varela, 2016). In winemaking, there has been a re-evaluation of the role of non-*Saccharomyces* yeast and their use in controlled mixed fermentations, with the main aim to enhance the analytical composition and aromatic profile of the final wine but also for other peculiar characters of these yeast species (Ciani et al., 2010; Benito et al., 2011; Comitini et al., 2011; Morata et al., 2012; Sadoudi et al., 2012; Quiros et al., 2014). Within the non-*Saccharomyces* yeast species, attention has been focused on *Torulaspora delbrueckii*, as this yeast has shown a positive impact in terms of low production of undesirable compounds, such as acetaldehyde, acetoin and acetic acid, and concomitant enhancement of other desired compounds (Bely et al., 2008; Comitini et al., 2011; Loira et al., 2012; Jolly et al., 2014; Azzolini et al., 2015). Indeed, the impact of non-conventional yeasts used in pure and mixed fermentations with *S. cerevisiae* on the flavor profile of other fermented and distilled spirit beverages, has been revaluated (Comitini et al., 2011; Jolly et al., 2014; Ciani and Comitini, 2015; Varela, 2016).

5 The New Tendency of Use of non-*Saccharomyces* in Beer Production

From the most ancient times, the production of beer was intended as a spontaneous fermentation with exposure to ambient atmosphere where the ‘contamination’ naturally transformed the raw materials into the final product. Gradually, spontaneous fermentation process has been transformed into a more controlled process, starting from the use of selected yeast strains belonging to the genus *Saccharomyces* expressly selected from different raw materials. Even today, 99% of the beers manufactured worldwide include modern beer styles still derived from fermentation processes conducted by *Saccharomyces* species (Bokulich et al., 2013).

Indeed, according to Basso et al. (2016), the use of *Saccharomyces* strains in controlled fermentations over decades is winning for the effective production of high ethanol, for the positive Crabtree effect and the higher tolerance to ethanol and other environmental stresses (Steensels et al., 2014).

However, the involvement of other species different to *Saccharomyces* species, already reported in some peculiar beer styles produced by spontaneous fermentations is coming back into vogue. Following this trend, non-*Saccharomyces* species can be used in a controlled manner for specific typologies as well as low-alcohol, alcohol-free beers or functional beers (De Francesco et al., 2015; Michel et al., 2016; Marco et al., 2017). In recent years, another very fashionable application of non-*Saccharomyces* yeasts is related to their use during controlled fermentations together with conventional *S. cerevisiae* starters (Ciani and Comitini, 2018). This practice has gained fast popularity among brewers especially to obtain distinctive products, with peculiar aromatic and flavor components (Vanderhaegen et al., 2006). Indeed, also if the choice of raw materials is crucial to achieve a quality product (Bernstein, 2010; Osbun et al., 2016), distinctive yeasts selected not only for their fermentation efficiency but also for the adjunctive aromatic properties are proving to be a winning choice. Several studies proposed the isolation of new starter yeasts from natural matrices (Marongiu et al., 2015; Mascia et al., 2015), and there was a development of microbrewery that supported the use of different yeast genera, with distinct impacts on flavor.

To achieve this, non-*Saccharomyces* yeasts may provide a new range of perspectives, representing a large source of biodiversity with consequent wide range of the fermentation products able to generate new beer styles. Indeed, within the wide non-*Saccharomyces* yeasts group, various genera and species have been proposed.

T. delbrueckii strains, widely studied in winemaking, have traditionally been used in the production of German style wheat beers (Hefeweizen) for their banana, bubblegum and clove-like flavors (Tataridis et al., 2013). In wine, *T. delbrueckii* yeast strains produce noticeably higher concentration of higher alcohols, esters, terpenes and phenolic aldehydes as well as other molecules like 2-phenylethanol, linalool, and methylvanillin (Ciani et al., 2010), which impart a distinct floral and fruity aroma and add to the sensory complexity giving a ‘wild/natural’ fermentation effect.

For this reason, it was also evaluated for beer production, in both pure and in mixed cultures with different *S. cerevisiae* starter strains (Canonico et al., 2016, 2017). The use of *T. delbrueckii* in pure cultures was characterized by a distinguishing analytical, aromatic profile and low alcohol content (2.66%; v/v) (Canonico et al., 2016). Generally, *T. delbrueckii* affected the aromatic compounds with the production of some fruity esters. Investigating on the possible use in beer production of *T. delbrueckii*, Michel et al. (2016) found that a strain was able to produce amyl alcohols as well as fruity and floral aroma. Furthermore, two strains were found to be suitable for producing low-alcohol beer owing to their inability to ferment maltose and maltotriose but still produced good flavor.

Lachancea termotholerans is a yeast species including strains with variable capability to ferment maltose. Domizio et al. (2016) found a strain of *L. thermotolerans* able to ferment wort and to increase the production of glycerol, lactic acid and decrease the pH value in comparison with *S. cerevisiae* starter strain. For this, the use of *L. thermotolerans* in pure culture to producing sour beers without the use of lactic acid bacteria was proposed. In this regard, Osburn et al. (2018) found that *Lachancea fermentati, Schizosaccharomyces japonicus* and *Hanseniaspora vineae* exhibited a sour character. Specifically, *Hanseniaspora vineae* strain exhibited high levels of attenuation after only 2 weeks and the final product was slightly sour but clean and highly drinkable, with notes of apple cider. The same authors found that also *Wickerhamomyces anomalus* was capable of heterolactic fermentation of sugar into lactic acid, ethanol, and CO_2. For this, brewing process in a large scale with these four non-*Saccharomyces* strains demonstrated that these yeasts have an appreciable effect to produce pleasant aromatic and flavor compounds (Osburn et al., 2018).

Saccharomycodes ludwigii is a species that does not utilize maltose or maltotriose in beer wort but ferments only glucose, fructose, and sucrose. For these reasons, it can be successfully employed low-alcohol or alcohol-free beers (De Francesco et al., 2015; Petruzzi et al., 2016).

Several authors (Sohrabvandi et al., 2010; Mohammadi et al., 2011; Mortazavian et al., 2014; Sarens and Swiegers, 2014) proposed *Zigosaccharomyces rouxii* and *Pichi*a spp. to produce low alcohol beers that were characterized by a distinctive flavor and reduced ethanol content.

The central point related to the use of non-*Saccharomyces* during beer fermentation is with regard to the enhancement of flavor and aroma complexity. The non-conventional yeasts proposed and their impact on beer production are summarized in Table 19.2.

TABLE 19.2

The impact of non-conventional yeasts on beer

Non-conventional yeast	**Impact on beer**	**References**
T. delbrueckii	Low alcohol content; Fruity esters	Canonico et al., 2016, 2017; Michel et al., 2016
L. thermotholerans	Low pH value Increase glycerol content Increase lactic acid	Domizio et al., 2016
Lachancea fermentati,	Sour character	Osburn et al., 2018
Schizosaccharomyces japonicus	Sour character	Osburn et al., 2018
Hanseniaspora vineae	Sour character	Osburn et al., 2018
Hanseniaspora vineae	Notes of apple cider	Osburn et al., 2018
Saccharomycodes ludwigii	Low-alcohol or alcohol-free beers	De Francesco et al., 2015; Petruzzi et al., 2016
Zigosaccharomyces rouxii	Low alcohol; increases flavor profile	Sohrabvandi et al., 2010; Mohammadi et al., 2011; Mortazavian et al., 2014; Sarens and Swiegers, 2014
*Pichi*a spp.	Low alcohol; increases flavor profile	Sohrabvandi et al., 2010; Mohammadi et al., 2011; Mortazavian et al., 2014; Sarens and Swiegers, 2014

6 Conclusions

Fermented beverages such as beer, as some of the oldest biotechnological fermentations, has been consumed by humans for thousands of years. Despite the discovery that these fermented beverages tasted good, it must have taken many years for humans to understand the mechanism behind the fermentation process, mainly regarding the microbiological aspect. Only at the end of nineteenth century, Louis Pasteur, considered one of the founders of microbiology, demonstrated the growth of yeast and its capacity to cause fermentation in a synthetic medium; this observation invalidated the concept that yeast, or ferments, originated from the action of oxygen on the nitrogenous elements of the fermentable liquid. From that time until now, the applied research, supported by innovative and advanced techniques, have reached significant advances, useful to manage the fermentation process.

Certainly, the fermented industry in general and beer production as consequence has changed dramatically in the past 50 years. The size of production capacity and consequent productivity has increased several folds: small and traditional facilities, as many microbreweries and small organic winery underwent a drastic reduction. Therefore, the fermented beverages industry is now dominated by big producers, moved from the only economic and profitable purpose. For this reason, the attention was focused on the standardization of the technological process that was managed to increase the production. In the other way, conscious consumers seek quality products in the market that meet non-industrial requirements. Recently in brewing, there was a worldwide growth of microbrewery that have introduced into the market niche beer products with peculiar and distinctive characteristics. This trend reinforced and encouraged the selection and the use of different yeast genera, with pronounced impacts on aroma and flavor. For this, the involvement of non-*Saccharomyces* yeasts in controlled mixed fermentation with *S. cerevisiae* starter cultures could be a practical way to improve the complexity and enhance the characteristics of beer.

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Cider Production Techniques in North America and Europe

Nazar Reehana
P.G. and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli, India

Mohamed Yousuff Mohamed Imran
P.G. and Research Department of Microbiology and Biotechnology, National College (Autonomous), Tiruchirappalli, India

Nooruddin Thajuddin and Dharumadurai Dhanasekaran
Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India

History

Cider drinking has been an American tradition since the Pilgrim's arrival in 1620, as cider was aboard the Mayflower. Barrels of cider could be found in the cellars of almost every farmhouse and city home in New England by 1838. Cider became the most popular drink in America by 1892 and remained the most widely consumed alcoholic beverage prior to its prohibition.

Very few domestic ciders were commercially available until about 1990. Only imported ciders from England and France, two regions that have been producing cider for centuries, were commercially available. While these imported ciders are quite popular in Europe, they are unable to make a strong impact in the American market because of their tannic and somewhat bitter flavor.

There are now dozens of commercially produced domestic ciders, and this number is expected to increase substantially. American ciders are made from sweeter, less tannic apples commonly grown in this country. The ciders from these varieties produce a fruity, somewhat sweeter, less tannic flavor than the European ciders and have been extremely successful in the United States.

- Since thousands of years the apple was used as a symbol for
  - Seduction (story of Adam and Eve)
  - Power, Dominance, Governance, and Authority
  - Various symbols in legends, myths, and fairy tales

Appearance and Types

The flavor of cider varies and can be classified from dry to sweet. Its appearance ranges from cloudy with sediment to completely clear, and color ranges from almost colorless to amber to brown. The variations in clarity and color are mostly due to the filtering between pressing and fermentation. Some apple varieties produce a clear cider without any need for filtration. Ciders are available in two forms as sparkling and still, the sparkling variety is the more common one.

Modern, mass-produced ciders closely resemble sparkling wine in their appearance. More traditional brands tend to be darker and cloudier. They are often stronger than the mass-produced varieties and taste more strongly of apples. Almost colorless, white cider has the same apple juice content as the conventional cider but is harder to create because the cider maker has to blend various apples to create a clearer liquid. White ciders tend to be sweeter and more refreshing. They are typically 7–8% ABV in strength. Black cider, by contrast, is a dry amber premium cider, which has an alcohol content of 7–8% ABV. The descriptor black usually comes after the brand name, such as Union Black and Barnstormer Black.

Major Cider Styles

Cider is the fermented apple juice, while perry is the fermented pear juice. There are two categories for ciders and perries: standard and specialty. The standard category covers ciders and perries made primarily or entirely from the juice of apples or pears (but not both at once). The only adjunct permitted in the standard category and only in some sub-categories, is a limited addition of sugar to achieve a suitable starting gravity or to raise sweetness post fermentation. Note that honey is not a ‘sugar’ for this purpose; a cider made with added honey must be entered either as a specialty cider or as a cyser under the appropriate mead sub-category. Other sugar sources that also add significant flavors (brown sugar, molasses) would also create a specialty cider (such as New England style).

A. Standard Cider and Perry Aroma

Apples (pears, if a perry) should be distinctive and dominate. There may be some fermentation by-products, such as esters, alcohols, and low levels of sulfur.

Appearance:
Color of the cider may vary from pale yellow to amber; appearance is clear and brilliant. Carbonation may vary from absolutely still to very vigorous as follows, entrant must specify still or carbonated (level of carbonation optional):

- Still : No carbonation visible or in the mouthfeel.
- Petillant : Very lightly sparkling, visibly and in the mouth.
- Sparkling : Clearly but not heavily carbonated.
- Spuming (or) Spumante : Heavily and vigorously carbonated, bordering on gushing, with tight, fine bubbles, champagne-like.

Flavor:
Crisp apple (pear) flavor should be present and distinctive; can be dry to sweet. Some noticeable alcoholic character may be present at the upper end of the range (7%). There should be a balance between the acidic character and the residual sweetness.

Mouthfeel: Light body; no astringency; no carbonic bite from CO_2.

Commercial examples:
Broadoak, Hecks, Dunkerton's, Franklins, Rich's Framhouse Cider (all available only in England), Clos Normand, Herout Fils, Hornsby's Draft Cider (not the 'Granny Smith' or 'Amber'), Sidra El Gaitero, Kelly's Traditional Irish Premium Hard Cider, Minchew Perry (available only in England), Wyder's Pear Cider.

B. Specialty Cider and Perry

Specialty cider/perry includes beverages made with added flavorings (spices and/or other fruits), those made with substantial amounts of sugar sources to increase starting gravities, and the beverage made from a combination of apple and pear juice (sometimes called pider). The same general characteristics and fault descriptions apply to specialty ciders as to standard ciders (preceding category), with the exception of added ingredients allowed.

Aroma:
Apples (pears) should be distinctive and dominate. There may be some fermentation by-products, such as esters, alcohols, and low levels of sulfur. Aromas from identified fruits and spices should also be noticeable.

Appearance:
Carbonation may vary from absolutely still to very vigorous. It is pale yellow in color, except where adjuncts such as spices or fruit may introduce a deeper shade or another color. Clear and brilliant.

Flavor:
Crisp apple (pear) flavor should be present and distinctive. Declared adjuncts must be present in the taste and integrate well with the base cider; can be dry to sweet. Some noticeable alcohol character may be present but the emphasis should be on alcoholic warming, not the taste or harsh bite of alcohol in the mouth. There should be a balance between the acidic character and the residual sweetness.

- May be flavored with spices.
- May have additions of sugar or honey.
- May have some type of fruit addition.
- If honey is the dominant fermentable, the entry is a cyser and must be entered as a mead.

Mouthfeel: Light to full body.

Ingredients: At least 75% apple (pear) juice with the remainder made from any variety of adjuncts. The alcohol content must be below 14%, but any type of yeast can be used in the production.

Commercial examples: Cider Jack fruit ciders.

C. New England-style Cider

This is a cider made with characteristic New England apples for relatively high acidity, with additives to raise alcohol levels and contribute additional flavor notes.

Aroma:
This cider has strong, pronounced apple aroma. The higher level of alcohol, i.e., 8–14%, will be more noticeable in the aroma. Other fermentation by-products may also be present.

Appearance:
This cider is pale to medium yellow in color; still or sparkling. Carbonation must be natural. It should be clear and brilliant.

Flavor:
This cider has strong apple flavor; usually dry; no hot alcohol taste. New England-style cider is distinguished from other styles by its robust and sometimes unsophisticated taste. It is a rustic, homemade product, typically more forceful than delicate. Nevertheless, complexity and structure are often present.

The fermentation of New England Barrel Cider

  - Occurs in barrels
  - Sweeteners added to increase alcohol are
    - Sugar
    - Boiled cider concentrate
    - Honey
    - Molasses or maple syrup (not recommended)
- Naturally fermented by adding raisins
  - Extended ferment in barrels (4 to 6 months)
  - Bottled in middle of March on a clear day
  - Draft cider is stored in wood and not bottled
    - Avoid exposure to air, which will cause acidification

Mouthfeel: Medium to full-bodied with some tannins.
Commercial examples: There are no known commercial examples of New England-style cider.
Processing:

- Good cider is made of about 30–40 different sorts of apples.
- Cider has about 5.5 to 7% of alcohol.
- 50 kg of apples make about 30 to 35 liters of cider.

The Manufacturing Process

The cider making process typically involves three stages, including crushing the fruit, pressing out the juice, and allowing it to ferment. To begin however, the fruit must be harvested, sorted, and washed.

Harvesting

The apples were picked by hand and transferred to large storage bins, which can hold about half a ton of fruit. When these bins are filled, they are transported by tractor to the processing plant. At the plant they are stored outside for about a week, which allows them to soften. This makes the apples easier to process and increases the amount of sugar in the juice.

Most ciders are a blend of juices from several apple varieties. A representative blend of apples would include approximately 50% sweet apples, 35% acidic apples, and 5% astringent apples (National Honey Board 2003). Although the juice from any apple can be made into cider, the cider makers suggest that the apple to be used should have certain characteristics: relatively high sugar content for alcohol production, a fibrous tissue to facilitate juice extraction, tannin to provide a bitter or astringent taste, and a pleasant apple taste and aroma (Cone 1997). For cider making, cider apples with more than 115 grams of sugar per liter of juice, and low amount of nitrogenous material should be used (Proulx and Cider 2003). According to Long Ashton Research Station (Proulx and Cider 2003), where the world's primary cider research is carried on, cider apples are classified as follows:

TABLE 20.1

Cider apples' classification on the basis of acid and tannin content

Apple group	Acidity (G Malic Acid/100 ml)	Tannin (G/100 ml)
Sweet	<0.45	<0.2
Bittersweet	<0.45	>0.2
Sharp	>0.45	<0.2
Bittersharp	>0.45	>0.2

Source (Proulx and Cider 2003)

Washing

After the apples have mellowed, they must be washed to remove leaves, twigs, insects, spray residues, and harmful bacteria. To this end, they are automatically poured out from the bins onto a scrubber. This machine rinses and scrubs each apple, removing most of the chemical residues from the skin. From there, they are moved along a conveyor to a hopper filled with water. The apples flow into the bath, which makes sure that they are thoroughly washed.

From the hopper, the apples are put on a conveyor and moved. Jets of water aid in moving the apples. During the manufacturing process, only whole apples are used because they have not been exposed to the flavor-damaging effects of oxidation. This means that each apple is inspected and any rotten or moldy fruits are removed. Since cider taste can be negatively affected by many different factors, cleanliness is essential during manufacturing.

Grinding

Next, the apples are put in a large mill and ground to a fine pulp with the consistency of apple sauce. This is done to ensure that the maximum amount of juice can be extracted from the apples. The finer the pulp, the greater the yield of juice. Fine grinding has the added benefit of reducing any damage caused by oxidation. The pulp is put into appropriately labeled 55 gal steel drums with plastic liners. Some of these drums continue on through the cider-making process while the rest are sent to a freezer to be used later. The frozen pulp ensures that cider can be produced throughout the year, even when apple supplies are low.

Pressing

To remove the juice from the pulp or pomace, it is pressed. Depending on the desired cider flavor, the pomaces from various types of apple pulp are used. Typically, anywhere from three to six different types are blended together in a large tank. This blend is then taken by the press operator and stacked for pressing. Wooden racks and forms are used for stacking the pomace. Each form is lined with a nylon cloth. Nylon is used because it is easy to clean and study enough to withstand many pressings. To start, several barrels of pomace are poured onto the cloth. The corners are then folded up and the form is removed. As a result, a square-shaped layer of pomace called a cheese is formed. A rack is placed on top of the cheese and another form is put in place. The process is repeated until 10–12 cheeses are in a stack. The whole stack is put in a large stainless steel tray that has been designed to hold the cider as it is pressed from the pomace. A worker puts the stack under the cider press, called a wring, and turns it on. This delivers as much as 30,000 lb (13,620 kg) of pressure from a hydraulic pump.

Blending

A well-balanced cider is a cider that includes different varieties of apples.

Blending can be done at any of the following steps:

1) Before grinding
2) Before fermentation—after pressing
3) After fermentation—blending at this step gives good control over the quality of the finished cider.

Cooling and Filling

The cider is expelled from the pomace and pumped to a cooling tank via plastic tubes. As the cider is transferred to the cooling tanks, it is passed through a screen mesh to remove any pulp pieces from the liquid. It is then chilled and stored at 33°F (0.6°C). This helps to inhibit the contamination by undesirable microorganisms. If this cider is of the unfermented variety, meant to be unfermented, it is sent to a mixing tank and pasteurized. Preservatives such as potassium sorbate are added and the juice is sent off to the filling lines.

Fermentation

Before fermentation, the various fermentation-assisting chemicals are added. Depending on the manufacturer, the cider may be allowed to ferment in a large, sealed bulk tank or in the individual bottles. If it is fermented in the bottles, the product will be sold with a bottom layer of sediment. The sediment is the remains of the fermentation yeast. In bulk fermentation, the cider is siphoned off after the yeast has died. This allows for a sediment-free product. Complete fermentation may take 1 month or more.

Natural fermentation of cider takes place in two stages:

1) The yeast flora is 'fed' by the natural hexose sugars and the products of fermentation are: alcohol and carbon dioxide (CO_2). Depending on the temperature and the juice constituents, this type of fermentation can last for weeks or even months as long as the sugar is available to the yeast. When sugar is no longer available for yeast, the fermentation slows down and then it stops.
2) The lactic acid bacteria ferment the natural malic acid found in apples into CO_2 and lactic acid. A malolactic fermentation (usually occurs spontaneously) is required if the cider is harsh and acidic at the end of the first fermentation; it also results in a smoother and gentle cider. However, while malolactic fermentation may be helpful with European apples, Michigan desert apple fermentations do not gain much from this step.

TABLE 20.2

Proportions of juice used in cider

Juice type	Percent of juice total
Neutral base	30–60
Tart	10–20
Aromatic	10–20
Astringent	5–20

Source: (Proulx and Cider 2003).

In terms of chemical reactions, alcoholic fermentation can be described as a three-step process (Margalit 1996):

a) Glucose and fructose (six carbon molecules) are split into phosphoglyceraldehyde (three carbon molecules) by phosphorylation.
b) Phosphoglyceraldehyde (three carbon molecules) is transformed into carbon acetaldehyde and carbon dioxide (source of CO_2 for fermentation) by decarboxylation.
c) Acetaldehyde is reduced to ethyl alcohol as an end product.

Most of the UK cider makers are of the view that a complete 'dry' fermentation, i.e., 10–12% alcohol, in as little as 2 weeks is a desirable objective (Lea and Drileau 2003). Incomplete fermentation can be obtained by removing the yeast halfway through the process, thus retaining less alcohol and more fermentable sugars than 'dry' hard ciders. In the US, commercial hard ciders usually contain about 5.5% alcohol and most are carbonated (Proulx and Cider 2003).

Fermentation is temperature-dependent. The first law to bear in mind is that the transformation of sugar gets faster with a rise in temperature. For example, wine fermentation is much faster at 30ºC than at 25ºC and at 25ºC than at 20ºC, and its speed doubles for each 10ºC variation (Peynaud 1984).

The second fermentation (malolactic fermentation) converts L(−)-malic acid to L(+)- lactic acid and carbon dioxide, and is carried out by lactic acid bacteria present in the apple juice. The malolactic fermentation can occur concurrently with the yeast fermentation but more often it is delayed until a few months later. However, hard cider prepared from typical Michigan desert apple varieties do not benefit much from malolactic fermentation.

Yeasts

Since the 1980s, specific cultured yeasts are being used in cider making. Inoculum mixes of active dried wine yeasts of *S. uvarum* and *S. bayanus* are widely used, as the former provides a speedy start while the latter copes better with fermentations to dryness since some yeast types are not tolerant to high alcohol concentrations (Lea and Drileau 2003). The vitality and viability of cultured cider yeasts under high stress conditions (i.e., higher alcohol concentrations and different types of alcohol) have recently been investigated.

If the cider is prepared traditionally, no additional yeast will be required. The internal fruit microflora together with the inoculum obtained from pressing cloths and equipment can give sufficient yeast counts to initiate fermentation within few hours if the temperature of the juice is above 10ºC (Lea and Drileau 2003). Hence, in a traditional cider fermentation, where no yeast is added and no sulfite is used, the first few days are dominated by the non-*Saccharomyces* spp., which multiply quickly to produce a rapid development of gas and alcohol. As the alcohol level rises (2–4%), these species die out and their place is taken over by other *Saccharomyces* spp., which complete the conversion of all the sugars to alcohol and generates a more wine-like flavor. Once all the available sugar is consumed, the final alcohol level is unlikely to exceed 8% from single-strength juice (non-concentrated).

Saccharomyces spp. are yeast species present in the juice mostly due to the contamination from the press cloths and factory equipment. They are also found on the skins of apples. In addition, these yeast species generate a distinctive range of flavors (Lea and Drileau 2003). In the commercial cideries, where the equipment is disinfected with chemicals to defeat the bacteria that cause cider sickness, the chance of natural fermentation is low because yeasts are killed at the same time with the unwanted bacteria. The same situation is encountered when a cider maker uses new equipment and a small amount of apples for making cider for the first time. Therefore, the addition of laboratory-grown 'wine-yeast' of known, reliable characteristics is necessary.

Temperature profile of the cider fermentation is strongly linked with yeast population dynamics of the predominant yeast species, present within the fermentation (Morrissey et al. 2004). In a recent study on Irish hard cider (Morrissey et al. 2004), it was reported that very rapid fermentation could be obtained in early season fermentation when ambient temperatures are higher with fresh 'must' temperatures of 14–16°C and higher yeast numbers present. In addition, rapid increase in sugar utilization, temperature rise, and ethanol production observed between days 4 and 8 were accompanied by rapid growth of *Saccharomyces* species during the same period. Lower temperatures that can be reached later in the production season can determine much longer fermentation with very different yeast profiles. Fermentation temperature is very important because it influences both the overall fermentation performance and the organoleptic properties of the final product.

Here are some examples of which yeast to use:

- champagne yeast
- wine yeast: Johanisberger, Tokay, Rhine; the most widely used yeast in North America is: Burgundy-descended 'Montrachet' yeast
- homemade yeast; raisins contain a lot of wine yeasts on their skins.
- yeasts starter—usually commercial white wine yeasts (dry or liquid) added for flavor purposes (to obtain champagne flavor)
- yeasts nutrients (ammonium and thiamine)—added at the same time with commercial yeast or when the fermentation slows down (sometimes this happens because of the low nitrogen levels in the cider).

The addition of yeast directly to the untreated juice may intensify the natural yeast, ending up with a good fermentation. However, the unwanted microorganisms will continue to increase in the cider and give serious trouble before the final product is ready, particularly if a low-acid juice is produced. In order to avoid these problems, many cider makers add sulfur dioxide (SO_2) to the juice before fermentation starts.

Sulfur dioxide (SO_2)—also named 'the sterilizer' is added to the freshly press juice before fermentation. Period of time to stand is 24 hours. Its role is to kill the bacteria and undesirable yeasts (the good yeast necessary for the fermentation process will survive).

Advantages of using SO_2:
Addition of SO_2 in cider making has some advantages: a good start with bacteria-free 'must', decrease loss of the batch, and a better fermentation. SO_2 is used as an antioxidant and an inhibitor of oxidizing enzymes. Furthermore, SO_2 combines with products of previous oxidation and prevents darkening (Herrero et al. 2003).

Disadvantages of using SO_2:
It is useful mentioning that some people are allergic to SO_2 at even very low concentrations. However, the use of SO_2 in cider making has been considered extremely necessary. Because of the health concerns, International Organizations (Joint FAO/WHO Expert Committee on Food Additives) have recommended its total elimination or at least reductions. Nevertheless, the legal permitted limit is 200 ppm (US Food and Drug Administration and European Commission). To achieve a better microbial stability without using SO_2, alternative methods have been proposed, such as apple juice storage under N_2 atmosphere (to avoid O_2 contact that is the cause of oxidation and microbial spoilage), the use of ascorbic acid, and yeast inoculation (Herrero et al. 2003).

Sugars—honey or white sugar may be added to the unfermented juices. Sugar is added to increase the amount of alcohol in the final product. Addition of sugar can be done at the same time with yeast and yeast nutrients or when the natural fermentation has settled. Information in Table 20.3 shows the amount of sugar necessary to be added to the juice.

The amount of natural sugar in the 'must' is measured with a hydrometer (used for measuring the specific gravity of soluble solids in the juice).

TABLE 20.3

Amount of carbohydrates necessary to increase specific gravity of the juice

Specific gravity increase	Additional sugar/gallon	Additional honey/gallon
5°	2¼ oz	3 oz
10°	4½ oz	6 oz
15°	6¾ oz	9 oz
20°	9 oz	12 oz

Source: (Proulx and Cider 2003).

Racking off

Using a clean plastic tube, the cider is drained off into the second fermenting tank or directly into the bottles. The prefermentation readings should be compared with these acid and alcohol levels.

Filtering (a) or Fining (b)

This step makes a cider crystal clear. It can be done by:

a) Using a closed filter system to avoid exposing the cider to air (risk of acetic bacteria contamination) or
b) Mixing gelatin, bentonite, and pectic enzyme into the cider.

Filling and Packaging

When the cider is ready for filling, it is filtered again and pumped into the appropriate packaging. In this filling process, the empty, sterile bottles move along a conveyor and are passed under a filling machine. The machine pumps cider into the bottles to the desired volume. The caps are then put on the bottles and then labeled. The jugs are put in boxes, then pallets, and stored at just an above-freezing temperature until the next day when they are delivered to the stores. There are several other additives that you can add to taste in your cider. The most common are acid blend, tannin, lactose, and corn sugar for carbonation. A bit of honey will help boost your alcohol content to make a strong cider. Light spicing with cinnamon or cloves can add a nice feel to your hard cider. Hard cider can be carbonated. Carbonation can occur as a result of natural process or it can be done artificially by dissolving carbon dioxide under pressure of 28 psi into the liquid. Carbonation is used to improve both the taste and 'texture' of the carbonated consumable. Carbonation is sometimes used for reasons other than consumption, to lower the pH (raise the hydrogen ion concentration) of a water solution.

Storage

The bottles should be kept in a cool, dark storage place for several months for flavor development.

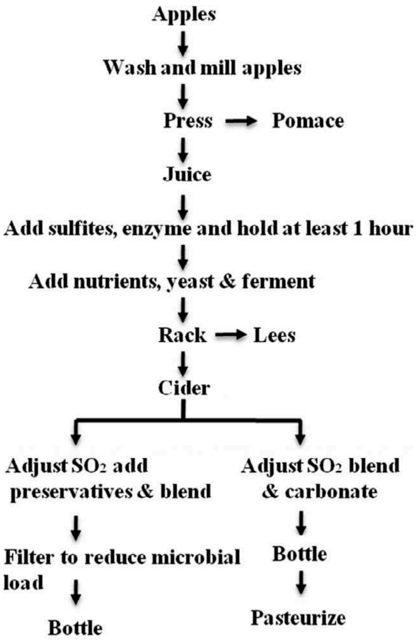
Apples
Wash and mill apples
Press
Pomace
Juice
Add sulfites, enzyme and hold at least 1 hour
Add nutrients, yeast & ferment
Rack
Lees
Cider
Adjust SO2 add preservatives & blend
Adjust SO2 blend & carbonate
Filter to reduce microbial load
Bottle
Bottle
Pasteurize

FIGURE 20.1 Flowchart of the typical steps and procedures involved in modern cider making.
Source: From Valois (2007).

Cider Tasting and Judging

Cider tasting can be defined in the same way as wine tasting: 'both an ancient art and a modern science' (Peynaud 1987). Like wine, cider tasting is essential for assessing quality during the process of making cider and for the purpose of buying and selling it. Cider tasting is the interpretation of a sum of sensations perceived either simultaneously or successively by the sense of sight, smell, and taste. These are activated by various sensory stimuli, and the touch- and temperature-sensitive receptors of the mouth are also involved (Peynaud 1987).

People perceive scents, flavors, and tastes differently, which is why describing those is not an easy job. People are linked to different sensory memories, and to different culturally acquired food habits.

The following attributes should be considered when evaluating cider:

Sight—the color of cider will vary with the apples used; the effervescence.

Smell—the aroma of the apples.

Touch—cider should have the right balance of malic acid and tannin.

Taste—the degree of sweetness.

Sound—range of effervescence (bubbles, carbonation).

Pathogenic and Spoilage Microorganisms in Cider

Bacterial pathogens such as *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* may occasionally occur in apple juice, being derived from the orchard soil, farm and processing equipment, or human sources. Outbreaks of food poisoning have occurred because of *E. coli* O157: H7 strains in freshly pressed nonpasteurized apple juice (usually known in the United States simply as cider). Normally, the acidity of both apple juice and fermented cider prevents the growth of pathogens, which survive for only a few hours. However, the specific strains of *E. coli* involved in food poisoning have a greater tolerance to acid and can survive for up to 30 days at 20°C in apple juice. These strains are destroyed by normal pasteurization conditions and do not survive in fermenting cider for more than 2–3 days because of the interaction of alcohol and acidity. The presence of bacterial endospores from species of *Bacillus* and *Clostridium* may be indicative of poor plant hygiene. They can survive for long periods and are frequently found in cider; however, because of its low pH value, they do not create a spoilage or health threat.

The juice from unsound fruits and juice contaminated within the pressing plant may show extensive contamination by microfungi, such as *Penicillium expansum*, *P. crustosum*, *Aspergillus niger*, *A. nidulans*, *A. fumigatus*, *Paecilomyces varioti*, *Byssochlamys fulva*, *Monascus ruber*, *Phialophora mustea*, and species of *Alternaria*, *Cladosporium*, *Botrytis*, *Oosporidium*, and *Fusarium*. None of these is of particular concern in cider making, except that spores of heat-resistant species, such as *Byssochlamys* spp., can survive pasteurization and grow in cider if it is not adequately carbonated.

The growth of *P. expansum* on apples leads to the occurrence of the mycotoxin patulin in the apple juice. Most countries have imposed a guideline limit of 50 µg l-1 for patulin. At high levels, patulin inhibits the yeasts used as starter cultures, but they metabolize the patulin under anaerobic fermentation conditions within a few days, to form a number of compounds, including ascladiol. Patulin, therefore, would not be expected to occur in cider unless patulin-contaminated juice was added to sweeten the fermented cider.

The role of organisms, such as *Brettanomyces* spp. and *Acetobacter xylinum*, in the spoilage of ciders during the latter stages of fermentation and maturation was mentioned previously. Of equal concern is the yeast *Saccharomycodes ludwigii*, which is often resistant to SO_2 levels as high as 1000–1500 mg l-1. *S. ludwigii* is an indigenous contaminant of cider-making facilities and can grow slowly during all the stages of fermentation and maturation. Its presence in bulk stocks of cider does not cause an overt problem. However, if it is able to contaminate ‘bright’ cider at bottling, its growth will result in a butyric flavor and the presence of flaky particles that spoil the appearance of the product. Although the organism is sensitive to pasteurization, it is known for contaminating the products at the packaging stage, either as a low-level contaminant of clean but nonsterile containers or directly from the packaging plant and its environment. Clumps of the organisms may also survive if it is present in unfiltered cider at the time of pasteurization.

Environmental contamination of final products with yeasts such as *Saccharomyces cerevisiae* vars. *cerevisiae*, *bailii* and *uvarum* can also occur. These will metabolize residual or added sugar to generate further alcohol and, more importantly, to increase the concentration of CO_2. Strains of these organisms are frequently resistant to SO_2. In bottles of bright cider inoculated with such fermentative organisms, carbonation pressures up to 900 kPa have been recorded. To avoid any risk of burst bottles, it is essential to maintain an adequate level of free SO_2 in the final product, particularly in multiserve containers that may be opened and then stored with a reduced volume of cider. Alternatively, a second preservative such as benzoic or sorbic acid can be used, where permitted by legislation. This precaution is not necessary for products packaged in single-serve cans and bottles, which receive a terminal pasteurization process after filling.

Conclusion

The society, culture, traditions, ethics, and food habits of any community cannot be seen in the isolated mode, as they are all intermingled and much more complex. The ecology provides a range of probability to select the plant and animal sources, culture decides the tradition, and the ethics determines the habit of eating the food. The traditions maintained by various communities of

Manipuris are having strong ethical issues. The advent of modern civilization has adversely affected the tradition and heritage of the community and thus the younger generations are not exposed to traditional practices. There should be focused efforts to promote traditional food systems within rural communities. Rewarding and acknowledging young people who demonstrate interest and awareness in the health of environment and in their traditional foods would also raise the profile of these issues. Traditional foods, community dinners can also provide opportunities to promote food products and knowledge about ethnic foods. In future, the nutritional and microbial aspects of various traditional foods are needed to be analyzed.

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21

Innovative Functional Fruit and Vegetable-Based Drinks Including Probiotics

Ilkin Yucel Sengun and Gulden Kilic
Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey

Introduction

Probiotics are live microorganisms which taken in sufficient amounts can provide health benefits. They are naturally found in the human and animal gastrointestinal system and breast milk. Lactic acid bacteria and *Bifidobacteria* are formed in the majority of probiotics and some other bacteria and yeast species may also exhibit probiotic properties. The main health benefits of probiotics are improvement of the immune system, treatment of ulcerative colitis, prevention of acute diarrhea and anticarcinogenic, and also they create antihypertensive, antimicrobial, antidiabetic, antiobesity, and antimutagenic effects.

Fermented products, which are produced from milk, cereal, meat, fruit, and vegetables, are important food sources of probiotics. In recent years, other than foods naturally containing probiotics, new probiotic foods are continuously developed in the food industry, including yogurt, cheese, milk, ice cream, fermented sausage, chocolate, and fruit-, vegetable- and cereal-based products. Dairy products are the most popular products among this group, but the consumption of them is limited for vegans and some people suffering from lactose intolerance and hypercholesterolemia. Therefore, it is very important to develop nondairy probiotic products as alternative foods for people especially who could not consume dairy origin foods. Fruits and vegetables could be used for developing nondairy probiotic foods and beverages, since they include high amount of nutrients, sugars, vitamins, polyphenols and dietary fibers which support the growth and survival of probiotics. Pineapple, cantaloupe, melon, cashew apple, apple, orange, tomato, and carrot are some of the products used for the production of probiotic beverages. In this chapter, the applications of probiotics in fruit and vegetable-based beverages are discussed in detail.

Probiotics

Probiotics are defined as “live microorganisms, affecting positively the gut microbiota, which taken in sufficient amounts can provide health benefits” by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (WHO/FAO 2002). This definition was also supported by the International Scientific Association for Probiotics and Prebiotics (ISAPP) along with Codex, European Food Safety Authority and Institute of Food Technologists (Hill *et al.* 2014). Probiotics contain a large range of microorganisms, mainly bacteria that belong to the genus *Lactobacillus* and *Bifidobacterium* (Burgain *et al.* 2011; Champagne *et al.* 2011; Saulnier *et al.* 2009). These microorganisms are naturally present in the human intestine. Other microorganisms identified as probiotic candidates are yeasts such as *Saccharomyces boulardii* and bacteria that belong to the genus *Bacillus*, *Bacteroides*, *Enterococcus*, *Escherichia*, and *Propionibacterium*. Commonly known probiotic microorganisms are given in Table 1.

These microorganisms, which are not typically found in foods, are commercialized as starter cultures (Prado *et al.* 2008; Vasiljevic and Shah 2008). Some criteria should be provided by microorganisms to describe them as probiotics (Berrada *et al.* 1991; Boyle *et al.* 2006; Collins *et al.* 1998; Da Cruz *et al.* 2007; Morelli 2000; Ouwehand and Vesterlund 2003; Pandey *et al.* 2015; Szajewska 2007) (Table 2).

The main health benefits of probiotics are given in Table 3. Probiotics can modify the intestinal microbiota by decreasing the pH, stimulating the immune system and producing antimicrobial effective metabolites (Agrawal 2007; Gerritsen *et al.* 2011; Zubillaga *et al.* 2001). *L. plantarum*, *L. rhamnosus* GG, and *Saccharomyces boulardii* are mostly used strains for the prevention of infections attached to *Clostridium difficile* (Koninkx and Malago 2008). Probiotics show inactivation effect on various microorganisms such as *Salmonella enterica* serovar Typhi, *C. difficile*, *Staphylococcus aureus, C. perfringens*, and *Escherichia coli* by producing short chain fatty acids, organic acids, bacteriocins and hydrogen peroxide (Kerry *et al.* 2018; Kober and Bowe 2015; Sagdic *et al.* 2004). Besides, they can be used to treat ulcerative colitis, Crohn's disease, gastric inflammation associated with *Helicobacter pylori* and enteric infections caused by *C. difficile*, *Listeria monocytogenes*, and *Salmonella* spp. (Kruis *et al.* 2004; Lesbros-Pantoflickova *et al.* 2007; Marteau *et al.* 2006; Prantera *et al.* 2002; Van Gossum *et al.* 2007).

Probiotics are mainly found in human and animal gastrointestinal system and also in breast milk (Gibson *et al.* 2017; Hill *et al.* 2014; Syngai *et al.* 2016). They have been firstly linked to dairy products, which are known to be the main sources of probiotics. In general, fermented products produced not only from milk but also based on cereals, meat, fruits and vegetables, are important sources of probiotics (Kandylis *et al.* 2016). In recent years, other than foods naturally

TABLE 1

Probiotic microorganisms

Genera	Species
Akkermansia	*A. muciniphila*
Bacillus	*B. cereus* Toyoi, *B. coagulans*, *B. laterosporus*, *B. subtilis*
Bacteroides	*B. uniformis*
Bifidobacterium	*B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. infantis*, *B. longum*, *B. thermophilum*
Enterococcus	*E. faecalis*, *E. faecium*
Escherichia	*E. coli* Nissle
Lactobacillus	*L. acidophilus*, *L. brevis*, *L. bulgaricus*, *L. casei*, *L. crispatus*, *L. curvatus*, *L. delbrueckii*, *L. fermentum*, *L. gasseri*, *L. johnsonii*, *L. lactis*, *L. helveticus*, *L. plantarum*, *L. paracasei*, *L. reuteri, L. rhamnosus*
Lactococcus	*L. lactis*
Pediococcus	*P. acidilactici*, *P. pentosaceus*
Peptostreptococcus	*P. productus*
Propionibacterium	*P. jensenii*, *P. freudenreichii*
Saccharomyces	*S. boulardii*
Streptococcus	*S. cremoris*, *S. diacetylactis*, *S. intermedius*, *S. salivarius, S. sanguis*, *S. oralis*, *S. mitis*, *S. thermophilus*

The table has been adapted from de Vrese and Schrezenmeir (2008); Rivera-Espinoza and Gallardo-Navarro (2010); Tripathi and Giri (2014) and Kerry *et al.* (2018).

TABLE 2

Characteristic properties of probiotics

Properties
Ability to influence metabolic activities
Ability to modulate the immune system
Ability to persist within the gastrointestinal tract
Adhesion to target epithelial tissue
Antigenotoxic property
Genetically stable
Human origin
Non-pathogenic
Production of antimicrobial metabolites
Resistance to technological processes
Resistance to pancreatic enzyme, acid and bile
Short generation time

TABLE 3

Effects of probiotics on health

Effectiveness	**Probiotics**	**References**
Anticancer	*L. acidophilus*, *L. casei*, *L. rhamnosus*	Aso and Akazan1992; Weill *et al.* 2013; Marinelli *et al.* 2017
Antidiabetic	*Lactobacillus* spp., *Bifidobacterium* spp.	Grover *et al.* 2012; Hampe and Roth 2017
Antimicrobial	*Lactococcus* sp. HY 449, *L. gasseri* SBT2055, *Bacillus subtilis*	Nishiyama *et al.* 2014; Kober and Bowe 2015; Upadhaya *et al.* 2016
Antiobesity	*L. gasseri, L. casei*, *L. acidophilus*, *B. longum*	Kang *et al.* 2013; Karimi *et al.* 2015
Effect on immune system	*L. acidophilus, L. johnsonii* LJ-1, *L. salivarius* UCC 118, *B. longum*	Saarela *et al.* 2000; Lollo *et al.* 2013
Prevention of acute diarrhea	*L. rhamnosus* GG, *S. boulardii*, *B. lactis*	Szajewska *et al.* 2001; Guandalini 2011
Prevention of *Helicobacter pylori* infection	*L. salivarius, L. casei* Shirota, *L. johnsonii* La1	Aiba *et al.* 1998; Gotteland and Cruchet 2003; Sgouras *et al.* 2004
Effect on respiratory system	*L. acidophilus* NCFM, *B. animalis* subsp. *lactis* Bi-07, *B. animalis* subsp. *lactis* BB-12	Leyer *et al.* 2009; Taipale *et al.* 2010
Secretion of bile salt hydrolase (BSH)	*Bifidobacterium* spp., *Lactobacillus* spp., *Pediococcus* spp.	Lye *et al.* 2010; Huang *et al.* 2013; Tsai *et al.* 2014
Effect on skin	*L. plantarum*, *Lactococcus* sp. HY 449	Oh *et al.* 2006; Takeda *et al.* 2014
Treatment of ulcerative colitis	*B. longum*	Fujimori *et al.* 2009

TABLE 4

Applications of probiotics in various food products

Food Products	**Probiotic**	**Reference**
Dairy Products		
Cheddar cheese	*L. acidophilus* 4962, *L. acidophilus* L10, *L. casei* 279, *L. casei* L26, *B. longum* 1941, *B. lactis* B94, *B. longum* 15708	Ong *et al.* 2006; Ong and Shah 2009; Fortin *et al.* 2011; Amine *et al.* 2014
Cheese and cheese-based product	*B. animalis* subsp. *lactis*	Cardarelli *et al.* 2008
Cheesecake	*L. rhamnosus* HN001	Sengun and Hasgucmen 2018
Fermented acerola ice cream	*B. longum* BL-04 *B. lactis* BL-01	Favaro-Trindade *et al.* 2006
Fruit flavored yogurt (enriched with strawberry, peach, banana)	*L. acidophilus* *L. casei* *L. paracasei* *L. rhamnosus* *Bifidobacterium* spp.	Vinderola *et al.* 2002
Ice cream	*L. acidophilus* *B. lactis*	Akin *et al.* 2007
Minas cheese	*L. acidophilus* LA5	Felicio *et al.* 2016
Mixed fruit yogurt	*L. acidophilus*	Kailasapathy *et al.* 2008
Soybean yogurt (enriched with mango and guava pulps)	*L. acidophilus* *B. animalis*	Bedani *et al.* 2014
Tofu	*L. casei* ŁOCK 0900, *L. paracasei* ŁOCK 0919	Zielińska *et al.* 2015
Yogurt (enriched with pea protein and fiber, chickpea and lentil flour, soy protein concentrate and soybean)	*L. acidophilus* *L. rhamnosus*	Zarea *et al.* 2012
Yogurt (enriched with skim milk, sodium caseinate and whey protein concentrate)	*S. thermophilus* *L. bulgaricus* *B. animalis* subsp. *lactis*	Marafon *et al.* 2011
Fruit-Vegetable Products		
Banana puree	*L. acidophilus* CCRC 10,695	Tsen *et al.* 2004
Chestnut puree	*L. casei* 491, *L. casei* 496, *L. rhamnosus* GG, *L. rhamnosus* VT1, *L. rhamnosus* RBM526, *L. rhamnosus* RBT739	Blatiotta *et al.* 2012
Fermented green olives	*L. paracasei* IMPC2	Sisto and Lavermicocca 2012
Green olives	*L. pentosus* B281, *L. plantarum* B282	Blana *et al.* 2014

(*Continued*)

TABLE 4 (Cont.)

Food Products	Probiotic	Reference
Pear puree	*Leuconostoc mesenteroides* 51-3	Kim *et al.* 2010
Sauerkraut	*L. paracasei* LMG P22043	Sarvan *et al.* 2013
Meat Products		
Dry fermented sausage, Fermented sausage	*L. casei* ŁOCK 0900 *L. fermentum* HL57, *L. paracasei* ŁOCK 0919, *L. plantarum* 299V, *L. rhamnosus* GG, *Pediococcus acidilactici* SP979	Ruiz-Moyano *et al.* 2011; Wójciak *et al.* 2012; Rubio *et al.* 2013
Dry fermented sausage	*L. reuteri* ATCC 53608	Muthukumarasamy and Holley 2006
Chocolate Products		
Dark chocolate	*L. rhamnosus* R0011, *Bacillus indicus* HU36	Raymond and Champagne 2015; Erdem *et al.* 2014
Milky and dark chocolate	*L. acidophilus* NCFM, *B. lactis* HN019	Lalicic-Petronijevic *et al.* 2015
Milky chocolate	*L. brevis* NTM003	Yonejima *et al.* 2015

containing probiotics, new probiotic foods are continuously developed in the food industry (Table 4), including yogurt, cheese, milk, ice cream, fermented sausage, chocolate, and fruit, vegetable and cereal-based products (Kandylis *et al.* 2016; Khan *et al.* 2014; Shori 2016; Tripathi and Giri 2014).

As can be seen from Table 4, most of the probiotic foods based on dairy products and probiotics mostly used in developing foods include *L. acidophilus*, *L. casei*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *B. breve*, *B. lactis*, *B. longum* and *B. animalis.*

Prebiotics

Gibson and Roberfroid (1995) defined prebiotics as "non-digestible food ingredients that selectively stimulate the growth and/or activity of one or a limited number of microbes in the colon resulting in documented health benefits when consumed in sufficient amounts". These food ingredients should have several characteristics such as represent a selective substrate for probiotics in colon stimulating their growth or activity, are neither hydrolyzed nor absorbed in the upper digestive tract, and are able to modify the intestinal microflora of colon (Bosscher *et al.* 2006). Fructooligosaccharides (FOS), galactooligosaccharides (GOS), polydextrose, isomoltooligosaccharides (IMO), lactilol, lactulose, fructans, b-glucans, and resistant starch are the types of prebiotics (Forssten *et al.* 2011; Siro *et al.* 2008). Inulin and inulin type FOS and GOS meet the requirements of the criteria to be prebiotics (de Vrese and Offick 2010; Gibson and Roberfroid 1995). Prebiotics such as GOS, FOS, and inulin have been determined to enhance the level of beneficial bacteria such as *Bifidobacteria.* FOS can be consumed by *Bifidobacteria*, owing to β-1, 2-glycosidase enzyme (de Wiele *et al.* 2004; Forssten *et al.* 2011). Although most prebiotics are usually accepted as "dietary fibers" all dietary fibers have not been characterized as prebiotic (Forssten *et al.* 2011).

Barley, chickpea, einkorn, maize, lentils, oats, rye, triticale, and wheat are important prebiotic sources. Prebiotic intake is also possible by the consumption of whole grains such as brown rice, millets, sorghums, and teff (Bultosa 2016). Artichokes, asparagus, bananas, garlic, onions, rye and wheat contain small quantity of FOS. Prebiotics could be used in the production of some foods such as drinks, crackers, yogurts, fermented milks, and cookies (Evangelisti and Restani 2011). Products developed for babies are commonly enriched with prebiotics to bring them similar to breast milk. Prebiotics act by preventing the cohering of pathogenic bacteria and preventing urinary tract infections in the child (Vandenplas 2002). Attachment of prebiotics in the food matrix can affect the texture and flavor of the food, so they could be used instead of fat components, which generally gives creaminess and softness to foods (Ranadheera *et al.* 2010). Meanwhile, it is also important how the addition of prebiotics influence the sensory properties of the food matrix. Incorporation of inulin as prebiotic in a fermented milk more quickly decrease the pH value of the product (Hardi and Slanac 2000). On the other hand, addition of FOS (4%) in yogurt does not change the acidity and pH value of yogurt (Zhu 2004).

Symbiotic foods, which contain probiotics and prebiotics together, affect human health in positive way. The effects of symbiotic foods are more than the foods involving only probiotics or prebiotics (Gmeiner *et al.* 2000; Gomes and Malcata 1999; Roberfroid *et al.* 1998; Schaafsma *et al.* 1998). The use of prebiotics and probiotics together in a single food improves probiotic viability during storage of the food and also through the passage of gastrointestinal tract, and provides an effective implantation of probiotics in the colon (Roberfroid *et al.* 1998). Synergistic effect between probiotics and prebiotics leads to inhibition of the growth of pathogenic microorganisms found in gut (Homayouni *et al.* 2008). It was reported that using oligofructose and inulin together with *L. plantarum* and *B. bifidum* increases the beneficial effect on health and inhibits the growth of pathogens such as *E. coli* O157:H7, *Campylobacter jejuni* and *S.* Enteritidis (Fooks and Gibson 2002). However, high production costs for symbiotic foods limit their production and consumption. The use of probiotics that is able to synthesize prebiotics, such as *Bifidobacteria* strains that can synthesize galactooligosaccharides, might overcome this restriction (Dumortier *et al.* 1990; Van Den Brook *et al.* 1999).

Functional Foods

The term "Functional food" was first used in Japan in the mid-1980s for the foods that have a positive effect on health by helping to reduce the risk of certain chronic diseases, in addition to its nutritive properties (Cencic and Chingwaru 2010; Chonan 2011; Stringheta *et al.* 2007). Functional food is defined by Foods for Specified Health Use (FOSHU) regulatory system in the American Dietetic Association (ADA) as "Food, that includes whole foods and fortified, enriched or enhanced foods, have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis, at active levels" (Prado *et al.* 2008; Staton *et al.* 2001). European Commission Concerted Action on Functional Food Science (FUFOSE) reported that functional food requires to have high nutritional values, performs one or more functional beneficial effect on the body, and reduces the risk of certain types of diseases (Evangelisti and Restani 2011). Functional food is also defined as

> A functional food is, or appears similar to a conventional food. It is part of a standard diet and is consumed on a regular basis, in normal quantities. It has proven health benefits that reduce the risk of specific chronic diseases or beneficially affect target functions beyond its basic nutritional functions.
>
> (Doyon and Labrecque 2008).

According to this definition, capsules and pills are not accepted as functional foods. The functional foods change the microbial composition of the gut and due to the gut health of the consumer, various functional food substances are produced in the food industry. Probiotics can be used as food additives to produce functional foods (Salmeron *et al.* 2009; von Wright 2005). For the gut health, three basic ingredients, such as prebiotics, probiotics, and secondary plant metabolites (such as polyphenol compounds), are usually added to develop functional foods (Puupponen-Piamia *et al.* 2003).

In recent years, consumers are showing increased interest to functional foods containing probiotics and bioactive compounds whose consumption can help to reduce or prevent the risk of diseases. Probiotics together with prebiotics, unsaturated fatty acids and phytonutrients complete the functional food ingredients (Horackova *et al.* 2018; Jankovic *et al.* 2010).

In recent years, an important achievement has been obtained in improving dairy products containing probiotics such as fermented milk, flavored milk, various cheeses, milk powder, frozen dairy desserts, sour cream, buttermilk, and whey-based beverages (Mohammadi and Mortazavian 2011). Although most of the functional foods containing probiotics and prebiotics are based on dairy products, currently consumers, who have some preferences such as being vegetarian and have some health problems such as lactose intolerance, high cholesterol, high fat and milk protein allergies, want to consume nondairy products (Granato *et al.* 2010a; Horackova *et al.* 2018; Prado *et al.* 2008; Vijaya Kumar *et al.* 2015). However, most of the foods are not suitable for probiotic growth due to their composition (low levels of amino acids and proteins contents, presence of phenolics, flavonoids and organic acids) (Nualkaekul *et al.* 2011). In addition to food composition, processing and storage conditions of food products may also affect the viability of probiotics. Addition of probiotics to food products may also influence the quality properties of the product, especially the sensory properties, mostly in negative way (Tripathi and Giri 2014).

In recent years, nondairy probiotic products such as fruit juices, cereal-based products, vegetarian products, baby foods, breakfast cereals, and confectionary products have been developed (Anekella and Orsat 2013; Chen and Mustapha 2012; Granato *et al.* 2010a; Gupta and Abu-Ghannam 2012; Lee and Salminen 1995; Mortazavian *et al.* 2010; Noorbakhsh *et al.* 2013; Rivera-Espinoza and Gallardo-Navarro 2010).

Probiotics Beverages

Food products containing probiotics are very popular in the Western developed countries. More than 78% of the current worldwide probiotic sales are produced from yogurt or/and milk (Granato *et al.* 2010b). Fermented milks, especially acidophilus milk and bifidus milk, are the most popular probiotic beverages in Western Europe and North America (Lebaka *et al.* 2018). Using probiotics in dairy products has promising features for producing functional beverages, since they have perfect conditions for protecting the viability of probiotics (Buriti *et al.* 2007a,b; Souza and Saad 2009; El-Dieb *et al.* 2012). However, nondairy probiotic beverages are cheaper than probiotic dairy products in developing countries and all over the world. Nondairy beverages especially cereal-based traditional products commonly contain various probiotics (Granato *et al.* 2010a, b; Gupta *et al.* 2010; Rathore *et al.* 2012; Ozturk *et al.* 2013). Beverages produced from various sources by probiotic addition are given in Table 5.

L. acidophilus, *L. plantarum* and *Bifidobacterium* spp. are commonly used probiotics in milk and cereal-based beverages. However, the number of nondairy probiotic beverages, such as fruit and vegetable beverages, has increased in recent years (Kandylis *et al.* 2016).

Probiotic Fruit and Vegetable-Based Beverages

Dairy products are the most popular products among probiotic foods, but the consumption of these products is limited for vegans and people suffering from lactose intolerance and hypercholesterolemia.

TABLE 5

Probiotic-added beverages

Beverages	Probiotic	Reference
Dairy Products		
Acidophilus milk	*L. acidophilus*	Yerlikaya 2014; Shiby and Mishra 2013
Acidophilus-Bifidus milk	*L. acidophilus,* *Bifidobacterium* spp.	Yerlikaya 2014
Bifidus milk	*B. bifidum, B. longum*	Yerlikaya 2014
Fermented milk	*S. thermophilus* TA040, *L. acidophilus* NFCM, *L. acidophilus* LA5, *B. animalis* subsp. *lactis* BB12, *Propionibacterium jensenii* 702	Lollo *et al.* 2013; Ranadheera *et al.* 2016
Cereal Products		
Boza	*L. casei* Shirota,	Ozturk *et al.* 2013
Fermented soy beverage	*L. casei* KN291	Zielińska *et al.* 2014
Malt beverage	*L. plantarum* NCIMB 8826, *L. acidophilus* NCIMB 8821,	Rathore *et al.* 2012
Oat-based beverage	*L. plantarum*	Gupta *et al.* 2010

Therefore, nondairy probiotic products are formed as an alternative for these people (Martins *et al.* 2013; Peres *et al.* 2012; Ranadheera *et al.* 2010). Fruits and vegetables, which include healthy substrates such as high amount of nutrients, sugars, vitamins, polyphenols and dietary fibers, are suitable substrates for developing nondairy probiotic beverages (Ding and Shah 2008; Granato *et al.* 2010a). Raw materials and probiotics used in developing fruit and vegetable-based beverages are given in Table 6.

There are two methods in producing probiotic fruit and vegetable-based beverages. In the first method, probiotic is directly added into beverages. This method is successfully applied if the microorganism is acid tolerant. In the second method, after adding probiotic, the beverage is fermented under specified conditions. During the fermentation period, probiotics lead to decrease in sugar content and production of some metabolites having antimicrobial properties such as bacteriocins, hydrogen peroxide and organic acids. Therefore, fermentation methods have some advantages over the first method including only addition of probiotics in foods (Pereira and Rodrigues 2018).

TABLE 6

Probiotic fruit and vegetable-based beverages

Raw material	Probiotic	Reference
Apple, pineapple, orange, red-fruit	*Lactobacillus reuteri* DSM 20016	Perricone *et al.* 2014
Beetroot	*L. acidophilus*	Rakin *et al.* 2007
Carrot	*Bifidobacterium lactis,* *B. bifidum*	Kun *et al.* 2008
Cashew	*L. johnsonii*	Vergara *et al.* 2010
Cataloupe	*L. casei* NRRL B-442	Fonteles *et al.* 2012
Cherry	*L. plantarum* ATCC20174, *L. casei* ATCC 393,	Nematollahi *et al.* 2016

(*Continued*)

TABLE 6 (Cont.)

Raw material	Probiotic	Reference
	L. rhamnosus ATCC 7469	
Honeydew melon	*L. casei* NCIMB 4114	Saw *et al.* 2011
Orange	*L. paracasei* ssp. *paracasei*	Da Costa *et al.* 2017
Orange, grapefruit, black currant, pineapple, pomegranate, cranberry, lemon	*L. plantarum*	Nualkaekul and Charalampopoulos 2011
Orange, pineapple, cranberry	*L. casei* DN 114 001, *L. rhamnosus* GG, *L. paracasei* NFBC 43338, *B. lactis* Bb-12	Sheehan *et al.* 2007
Pear	*L. acidophilus*	Ankolekar *et al.* 2012
Plum	*L. kefiranofaciens*, *Saccharomyces boulardii*	Sheela and Suganya 2012
Tomato	*L. casei*, *L. acidophilus*, *L. plantarum*, *L. delbrueckii*	Koh *et al.* 2010

Probiotic Fruit-Based Beverages

Fruit-based beverages have a good refreshing taste and are consumed by people of all age groups. Besides, there is a huge interest in developing fruit-based functional beverages containing probiotic (Tuorila and Cardello 2002). There are some studies investigating the potential of the production of probiotic fruit juices. In a study performed by Mousavi *et al.* (2011), viability of *L. acidophilus* DSMZ 20079*, L. plantarum* DSMZ 20174*, L. delbrueckii* DSMZ 20006, and *L. paracasei* DSMZ 15996 in fermented pomegranate juice was investigated. The results showed that the initial numbers (10^7 CFU/mL) were remained at maximum level for *L. plantarum* (3.6×10^8 CFU/mL) and *L. delbruekii* (3.9×10^8 CFU/mL) within 2 weeks of storage at 4 °C and then the numbers decreased dramatically after 4 weeks.

In other study, cashew apple juice was inoculated with *L. casei* NRRL B-442 at 7.48 log CFU/mL level and after the fermentation period at 30 °C for 16 hours, the viability of probiotic was investigated at 4 °C for 42 days. Results of the study showed that the counts of *L. casei* increased to 8 log CFU/mL during the storage period while brightness-yellowness increased and redness decreased. It was concluded that cashew apple juice produced with *L. casei* could be an alternative beverage fortified with probiotic (Pereira *et al.* 2011).

Soursop juice was produced by addition of *P. pentosaceus* LBF2 (10^7 CFU/mL) and fermentation at 37 °C for 72 h. The viable cell counts of *P. pentosaceus* LBF2 in soursop juice were found higher than 10^6 CFU/mL after 4 weeks at 4 °C while color and other sensory properties were also found to be acceptable (Akpeji *et al.* 2017).

In another study, viability of probiotic in pineapple and strawberry-apple juices was examined during 28 days at 8 °C and 20 °C. The juice samples were inoculated with *B. animalis* subsp. *lactis* Bb12 (10^8 CFU/mL) and fermented at 37°C for 24 h. After the fermentation period, the number of probiotic in pineapple and strawberry-apple juices was determined as 7.89 log CFU/mL and 6.91 log CFU/mL, respectively. The counts were decreased by 1.7 log CFU/mL in pineapple juices after 28 days of storage at 8 °C and 22 °C. In strawberry-apple juices, no live cells were detected at 22 °C after 14 days, while the cell number was determined as 3.5 log CFU/mL in samples stored at 8 °C for 28 days (Horackova *et al.* 2018).

Probiotic coconut water beverage using *L. casei* L4 was produced by fermentation at 35 °C for 48 h. The results showed that the cell number (9.47 log CFU/mL), vitamin B12 content (11.47 µg/mL), total phenolic content (72.1 µg GAE/mL) and antioxidant activity (58.4 and 69.2%) were increased during the fermentation period. Honey (15%) and coconut flavor addition has improved the sensory properties of the fermented coconut water beverage (Giri *et al.* 2018).

In another study, probiotic mango juice was produced using *L. acidophilus* (MTCC10307), *L. casei, L. delbrueckii* (MTCC911), *L. plantarum* (MTCC9511), and fermentation at 30 °C for 72 h under micro-aerophilic conditions. After the fermentation period, pH, acidity, and probiotic counts were determined as 3.2, 1.72% and 1.0×10^9 CFU/mL, respectively. At the end of the storage period (4 °C/4 weeks), the counts of *L. acidophilus, L. casei, L. delbrueckii* and *L. plantarum* were determined as 1.5×10^7, 1.0×10^6, 1.6×10^6 and 1.4×10^7 CFU/mL, respectively. It was concluded that *L. plantarum* was the fastest strain in using sugar and reducing the pH value of the product among other probiotics used (Reddy *et al.* 2015).

Probiotic Vegetable-Based Beverages

Vegetable-based probiotic beverages have a number of attractive advantages such as containing beneficial nutrients and presenting fruit juices that are perceived to be healthy and refreshing. However, the sensory properties of these products could be affected in a negative way especially when *Bifidobacteria* are used (Vinderola *et al.* 2017). There are various studies showing the suitability of using variety of probiotics in vegetable beverages. For example, Yoon et al. determined that beverages such as beet, cabbage, and tomato juice are suitable environments for *L. acidophilus*, *L. casei*, *L. delbrueckii*, and *L. plantarum* (Yoon *et al.* 2004, 2005, 2006).

Probiotic beetroot beverage was produced by *L. rhamnosus, L. plantarum*, and *L. delbrueckii* at 37 °C for 24 h. The antioxidant activity and total phenolic and flavonoid contents of fresh fruit juice were determined as 75.8%, 22.12%, and 3.61%, respectively, while these values were found as 78.1%, 24.32%, and 3.85% in probiotic beverages, respectively. The acidity and protein values were found to be higher in samples produced with the addition of probiotics than the samples that were produced excluding probiotics (Panghal *et al.* 2017).

In another study, viability of probiotics including *L. acidophilus* MTCC 10307, *L. plantarum* MTCC 9496, and *L. casei* MTCC 5381 were investigated in tomato juice. Probiotic inoculated tomato juice (2.47–2.49×10^8CFU/mL) was incubated at 37°C for 72 h. It was observed that pH and sugar content of the product decreased while the numbers of probiotics increased at the end of fermentation period (Kaur *et al.* 2016).

Juices of broccoli, cauliflower, red and white cabbage were fermented by *L. casei* and *L. paracasei* at 30 °C for 24 hours and stored at 4°C for 42 days (Fadhil and Akın 2016). The probiotics used in vegetable juices maintained at high level (approximately at 8 log CFU/mL) during the storage period.

Probiotic stability in fermented (37 °C for 8–9 h) vegetable juice of beet, carrot and celery was investigated during storage at 4 °C for 21 days. *L. acidophilus*, *L. casei* and *S. boulardii* were inoculated in juice samples at 6 log CFU/mL. At the end of the storage period, the counts of probiotics were determined as 7.65–8.50 log CFU/mL for *L. acidophilus* and *L. casei*, and 7.04–7.46 log CFU/mL for *S. boulardii*. Sensory acceptance of vegetable juices was increased by the addition of honey into final beverages (Profir *et al.* 2015).

Zandi *et al.* (2016) have studied on the production of functional beverages including the mixture of apple, beet and carrot juices and *L. casei* 1608 (1.5×10^6–1.5×10^7 CFU/mL). The mixture of juices (20%, 30%, and 40%) fermented at 37°C for 48 h were stored for 28 days at 4 °C. Although the sample including 40% fruit juice (14% apple, 12% beet, and 14% carrot) was found to contain the highest number of probiotic (approximately 10^6 CFU/mL), this sample was the most unfavorable juice in terms of sensory properties. It was also reported that malt extract or glucose may be added to the juice for improving the sensory properties.

These negative effects on the sensory properties of products could be linked with the high amounts of lactic acid, acetic acid and other organic acids produced by probiotics. Therefore, further studies should examine both the viability of probiotics and also the sensory properties of the product (Rathore *et al.* 2012).

Factors Affecting the Probiotic Viability in Products

The properties of probiotics such as stability during storage, adaptation to the processing environment and the effect on sensory properties should be considered during development of probiotic products (Champagne and Gardner 2008). Additionally, the biological properties of probiotics such as oxygen sensitivity, resistance to enzymes in the gastrointestinal system, antioxidative ability, sensitivity to phenolic compounds (because of amino acid hydrolyzation) and lysozymes, and adhesion to animal cells should be considered. Therefore, utilization of multiple strains would be beneficial since all properties cannot be seen in a single strain (Champagne and Gardner 2008).

The health benefits of probiotics mainly depend on their concentration in foods and their ability to survive under adverse conditions of the gastrointestinal system. Therefore, as the probiotic viability is strain-dependent (Do Espirito Santo *et al.* 2011; Corbo *et al.* 2014; Tripathi and Giri 2014), they should be at least 10^7 CFU/mL at the end of the shelf life of the product, which approximately corresponds to 10^9 CFU/portion (Nualkaekul and Charalampopoulos 2011).

To improve the stability of probiotics in fruit and vegetable beverages, various prebiotics such as cellulose and dietary fiber or some ingredients that have protective effect on cells could be used in beverages. It was reported that before fermentation with *L. acidophilus*, addition of brewer's yeast autolysate into beetroot and carrot juices increased the growth of *L. acidophilus* and decreased the fermentation time. Also, the viability of probiotic can take longer time for the products enriched with amino acids, antioxidants, minerals, and vitamins (Rakin *et al.* 2007). Hence, various factors affect the viability of probiotics in products, which mainly includes pH, acidity, oxygen, and other gases in the environment, temperature and composition of foods.

One of the most important properties of probiotics is to have an acid resistance. The pH value of the product is an important factor affecting the stability of probiotics during storage time. Fruit and vegetable-based beverages contain a high amount of organic acids, which affect the viability of cells in a negative way. Lactobacilli are more resistant to low pH values than other probiotics. *Bifidobacteria* are less acid tolerant and cannot survive at an approximate pH value of 4.6 (Reddy *et al.* 2015; Tripathi and Giri 2014). It is also possible that a microorganism capable of withstanding in the acidic foods may not able to resist the acidity of the stomach. For example, *L. lactis* and *S. thermophilus* grow well in acidic foods, but they cannot grow in the gastrointestinal tract and lose their probiotic functions. In contrast, a strain able to resist to the acidity of stomach may not able to resist the acidity of a product during the storage time (Galgano *et al.* 2015). Despite that the pH is a drawback for probiotic survival in juices, Ranadheera *et al.* (2014) observed that the incorporation of lactic acid bacteria into fruit juices with low pH increased the resistance of bacteria to successive stressful acidic conditions, such as those found in the gastrointestinal tract. When acidification is decreased, it provides the survival of probiotics at 25 °C for more than 9 days in orange juice (Sohail *et al.* 2012).

Most of the probiotics commonly grow under unaerobic or microaerobic conditions. Hence, the level of oxygen within the package during storage of foods should be as low as possible to prevent toxicity and growth of probiotics, but the extent of sensitivity is strongly variable. *Bifidobacteria* spp. could not tolerate oxygen while some of the species belonging to Lactobacilli can grow under aerobic conditions. Oxygen causes an oxidative damage on unaerobic cells by the formation of reactive oxygen species (ROS), such as superoxide ion or H_2O_2. Hence, the modification of product atmosphere such as increasing the content of CO_2 could be used as a tool to reduce the negative effects of oxygen on probiotics (Corbo *et al.* 2014; Gaudreau *et al.* 2013; Nag and Das 2013). Generally, *Lactobacillus* is more resistant to oxygen than *Bifidobacteria* (Nag and Das 2013). Some researchers evaluated the effects of different concentrations of (−) catechin, epigallocatechin gallate, green tea and green tea extracts (GTE) on the growth and survival of some probiotic strains such as *Leu. helveticus* with different oxygen sensitivities (Nag and Das 2013). Hence, using antioxidant compounds could be a good alternative to limit the negative effects of oxygen.

The viability of probiotics in fruit and vegetable beverages can be negatively affected from the storage temperatures, as the thermal exploitation could show a detrimental effect. The growth temperature for most of the probiotics ranges between 15 °C and 45 °C, while the optimum growth temperature ranges around 35°C (Boylston *et al.* 2004; Korbekandi *et al.* 2011). Some species like *L. acidophilus* can grow at temperatures as high as 45 °C, but the optimum growth occurs at 40–42 °C. Temperatures above 45–50 °C during food processing are harmful for the viability of probiotics. The exposure time should be shorter at high temperature in order to benefit from the probiotics. It is suggested to add probiotics after thermal treatment of food (Lee and Salminen 2009). Additionally, the resistance of probiotics to thermal stress can be increased by mild heat treatment before their use. Application of nonlethal heat shock allows bacteria to tolerate a second heat stress higher in intensity and it has been stated that the heat adaptation increases the thermal tolerance of probiotics such as in Lactobacilli (Teixeira *et al.* 1994). Microencapsulation of dried probiotics could be a way of preventing the cells against thermal treatment (Champagne and Gardner 2005).

Fruit and vegetable-based beverages, including minimum levels of protein and amino acids, and various compounds inhibiting the growth of microorganisms such as organic acids, phenolic and flavonoid compounds, may not be suitable for probiotic growth (Nualkaekul *et al.* 2011). In the study carried by Perricone *et al.* (2014), the viability of *L. reuteri* DSM 20016 in green apple, pineapple, red fruit and orange juices was investigated and it was observed that the viability of probiotic was decreased in red fruit juices because of the combined effects of phenols and low pH. On the contrary, in some studies, the viability of probiotic was increased when different amounts of red fruit juices or acids were used (Perricone *et al.* 2015; Saarela *et al.* 2011). Hence, the viability of probiotic was the outcome of synergistic and antagonistic action of some factors. Usually, low pH exerts a detrimental effect on cells, but dietary fiber and protein could protect them from acidic stress. On the other hand, the role of citric and malic acids is controversial, as they seemed to protect probiotics, whereas phenols could cause a strong viability loss (Tripathi and Giri 2014). Hence, some ingredients that support the viability of probiotics such as ascorbic acid, can be added to these beverages (Antunes *et al.* 2013; Martins *et al.* 2013; Nualkaekul and Charalampopoulos 2011).

Microencapsulation

Stability, survival and resistance to gastric juices and bile salts are essential for probiotics, since they can show their beneficial properties based on these qualities (Heidebach *et al.* 2012). In order to enhance the viability of probiotics, microencapsulation successfully creates physical protection during food storage and enables the passage of probiotic through the digestive tract. Microencapsulation is defined as “the technology of packaging of solid, liquid, or gaseous materials miniaturized in capsules that can release their contents in a controlled manner and only under certain conditions” (Burgain *et al.* 2011). This capsule containing the microorganisms is produced of a substance that forms a thin, resistant, semipermeable membrane, to protect the content during the passage into the stomach. Various materials can be used for microencapsulation such as plants (starch and gum arabic), polysaccharides derived from seaweed (carrageenan and alginate), animal protein (casein), and substances of bacterial origin (gellan and xanthan gum) (Galgano *et al.* 2015).

Microencapsulation technologies can be designed and practiced using a variety of matrices such as alginate and chitosan to prevent the probiotics from the damage induced by the external environment (Lee *et al.* 2004). Many studies showed that microencapsulation might supply a suitable anaerobic environment for oxygen-sensitive probiotics, and besides it forms a barrier against the acidic conditions of the fruit and vegetable juices such as sapodilla, orange, cherry, and tomato juices (Ding and Shah 2008; Gaanappriya *et al.* 2013; King *et al.* 2007; Nematollahi *et al.* 2016).

Conclusion

Over the past two decades, a considerable amount of research has been focused on development of probiotic foods. The results of the studies showed that fruits and vegetables, which include healthy substrates such as high amount of nutrients, sugars, vitamins, polyphenols and dietary fibers, are suitable environments for developing nondairy probiotic beverages as alternative functional foods for vegans and people have some limitation to consume dairy products. Probiotics such as *L. acidophilus*, *L. casei*, *L. fermentum*, *L. paracasei*, *L. plantarum*, *L. rhamnosus* GG, and *B. bifidum* have been commonly used in the development of many fruit and vegetable-based products. The juices of pineapple, pear, orange, melon, cashew apple, banana, beetroot, carrot, tomato are the products developed by addition of probiotics. However, the properties of probiotics such as oxygen sensitivity, resistance to enzymes in the gastrointestinal system, antioxidative ability, sensitivity to phenolic compounds and lysozymes, adaptation to the processing environment and the effect on sensory properties should be considered during the development of probiotic products. Microencapsulation might supply a suitable anaerobic environment for oxygen-sensitive probiotics, and besides it forms a barrier against the acidic conditions and thermal treatment of the fruit and vegetable juices. Hence, encapsulation technologies could be useful for improving probiotic cell viability and obtaining more stable probiotic products.

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Section 7

Molecular Tools in Fermented Food Products

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Molecular Methods in Fermented Foods

Kolawole Banwo
Department of Microbiology, University of Ibadan, Ibadan, Nigeria

Adekemi Titilayo Adesulu-Dahunsi
Department of Microbiology, Landmark University, Omu-Aran, Nigeria

Samuel Olatunde Dahunsi
Department of Microbiology, Landmark University, Omu-Aran, Nigeria

1.0 Introduction

Spontaneous fermentation involving microorganisms is an old method of food production, which aims at increasing the shelf life of the raw materials. It is a cost-effective method of food preservation for short- or long-term use. It involves the use of bacteria and yeast to convert carbohydrates to alcohol in the absence of oxygen (Edward et al. 2012). Not only does food fermentation function in the preservation of foods but it also helps retain the nutrient value present in the food (Olasupo et al. 1997). It causes volume reduction of food or raw material for easier transportation from one place to another and enhancing the nutritional value of the food products (Rebouçaset al. 2016). This food preservation technology has become a traditional practice among the populace in developing countries at household or village-level. Several African indigenous foods undergo fermentation before consumption. Microorganisms usually carry out the fermentation of these foods, especially bacteria and yeasts where they utilize the food as a substrate for their propagation (Gupta and Abu-Ghannam, 2012). Traditional microbial food fermentation plays numerous functions such as improvement of flavor, aroma, texture, preservation, and extending of the shelf life through lactic acid, acetic acid, or alkaline fermentation. In addition, enhancing the quality with the production of essential proteins, vitamins and amino acids, improving the digestibility, making nutrients available, and detoxification of anti-nutritional factors (Jimoh et al. 2012; Oyewole and Isah, 2012). Several studies on fermented food have revealed that they not only possess bio-preservative properties but also wield health benefits (Oyewole and Isah, 2012).

Several microorganisms that perform important functional roles in the production of fermented foods are un-culturable using only culture media. The culture-independent methods are universally employed in food microbiology to profile both culturable and non-culturable population dynamics of microorganisms from fermented foods (Cocolin and Ercolini, 2008a; Cocolin et al. 2013). Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) have been developed to obtain microbial populations straight from fermented foods, which are centered on sequencing of the specific regions of 16S rDNA and 26S rDNA amplicons produced by polymerase chain reaction (PCR) (Ercolini 2004; Alegría et al. 2012). Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis is one of the most popular culture-independent techniques used for the detection of microorganisms in fermented foods, which aids in reporting the bacterial and yeast populations. This technique assists in the study of both inter- and intra-species diversities among genus and genera (Cocolin et al. 2002; Tamang 2010;

Cocolin et al. 2011; Greppi et al. 2013a, 2013b). Molecular identification is a precise and reliable method, which employs both culture-dependent and culture-independent techniques for identification of microorganisms from fermented foods. Species-specific PCR techniques are employed for species-level identification (Tamang et al. 2005). Random amplified polymorphic DNA (RAPD-PCR) is a typing method based on the genomic DNA fragment profiles amplified by PCR and is usually used for discrimination among strains from fermented foods (Chao et al. 2008). The repetitive extragenic palindromic sequence (rep-PCR) technique allows the typing at subspecies level and indicates important differences among strains of the same species from fermented food samples (Banwo et al. 2012; Tamang et al. 2012; Adesulu-Dahunsi et al. 2017a, 2017b). Amplified fragment length polymorphism (AFLP) is a procedure based on the selective amplification and separation of genomic restriction fragments with discriminatory abilities in various microorganisms (Oguntoyinbo et al. 2007). Understanding the biodiversity of microbes involved in the fermentation of traditional African foods employing phenotypic and genotypic methods is very imperative to its sustainability.

2.0 Brief Ecology of Microorganisms Involved in African Indigenous Fermented Foods

2.0.1 Brief Ecology of *Bacillus* Species and Associated Microorganisms in Fermented African Vegetable Condiments

African vegetable condiments are products processed from raw materials such as seeds and leaves through spontaneous fermentation. These include *iru* (*soumbala, dawadawa*) from locust beans (*Parkia biglobosa*), *ogiri* from melon seeds (*Citrulus vulgaris*), *bikalga* from the seeds of roselle (*Hibiscus sabdariffa*), *maari* from baobab seeds (*Adansonia digitata*), *ugba* from African oil bean (*Pentaclethra macrophylla*), *owoh* from cotton seeds (*Gossypium hirsutum*), *okpehe* from mesquite seeds (*Prosopis africana*) (Odunfa 1981; Sanni and Ogbonna, 1991; Ouoba et al. 2003; Ouoba et al. 2008; Parkouda et al. 2009; Oguntoyinbo et al. 2010). These products undergo alkaline fermentation process are used as soup thickeners, condiments or as side dishes in many African countries. The microbial diversity associated with these products suggests different classes of bacteria, mainly of the aerobic mesophilic groups (Parkouda et al. 2009; Ouoba et al. 2008; Oguntoyinbo et al. 2010). Additional microorganisms associated with the fermentation of condiments include *Escherichia*, *Proteus, Pediococcus, Micrococcus, Staphylococcus, Streptococcus, Alcaligenes, Pseudomonas, Corynebacterium,* and *Enterococcus* species (Odunfa 1981; Oguntoyinbo et al. 2007). *Staphylococcus* spp. and *Micrococcus* spp. are very dynamic during the initial stages of the fermentation process by rapid multiplication within 24h of fermentation and then decrease as fermentation time progresses. Achi (1992) reported the co-dominance of *Staphylococcus* and *Bacillus* spp. as the microflora of fermenting beans and other vegetable condiments. *Staphylococcus* species have been associated with the fermentation of foods from plants, especially vegetable proteins (Odunfa and Komolafe, 1989; Omafuvbe et al. 2003). Members of the Enterobacteriaceae contribute to the microbial ecology of fermenting plant and vegetable proteins at the early stages of the fermentation process (Achi 1992). These microorganisms do not survive until the end of the fermentation process, probably because of the modified environment, which develops to alkaline at later stages. *Bacillus* spp. and related genera are the main bacteria responsible for the alkaline fermentation of African vegetable condiments. The predominant species are *Bacillus subtilis* while other species like *Bacillus pumilus, Bacillus megaterium, Bacillus licheniformis, Bacillus circulans, Bacillus cereus, Bacillus borstelensis, Paenibacillus polymyxa, Lysinibacillus sphaericus*, and *Lysinibacillus fusiforms* has been reported (Odunfa 1981; Sanni and Ogbonna, 1991, 1992; Ouoba et al. 2004; Parkouda et al. 2009; Oguntoyinbo et al. 2010). The predominance of these microorganisms in the foods/condiments is linked to the production process, which involves long hours of cooking of the raw materials, utensils, and handling

(Odunfa 1981; Ouoba et al. 2008). *Bacillus* species are responsible for the fermentation of alkaline foods due to the properties they possess such as formation of heat-resistant spores and ubiquitous nature. The endospores of these bacilli must have been associated with the cotyledons of the vegetables/plants from the onset of the fermentation. These microorganisms possess one or more hydrolytic enzyme properties, which is produced during the fermentation of the vegetable condiments (Sanni et al. 1999; Oguntoyinbo et al. 2007). However, *B. subtilis* is the most predominant microorganism with characteristics such as proteolytic, polyglutamic acid production, pyrazine, and antimicrobial such as subtiliosin production (Oguntoyinbo et al. 2007). The metabolic and enzymatic hydrolysis of *Bacillus* species aids in the breakdown of protein into amino acids (Oguntoyinbo et al. 2010).

Fermentation in any food matrix is caused by the development of interactions among several different microorganisms in a complex microbiological process. During the fermentation process, the microorganisms utilize nutritional constituents of the seeds, thereby transforming them into products that contribute to the biological composition and palatability of the condiment (Odunfa 1981; Achi 2005). A diverse microbial flora, which eventually is predominant of *Bacillus* group, mediates the production of fermented condiments (Odunfa 1985; Oguntoyinbo et al. 2007).

2.0.2 Brief Ecology of Lactic Acid Bacteria in Indigenous Fermented Foods and Beverages of Africa

In many countries of Africa, there are some traditional fermented foods known for production at the household level, which include *gari, ogi, fufu, masa, fura, kunun, burukutu,* and *pito.* These foods consist of starchy, dairy, cereal-based substrates and alcoholic beverages. Most of these foods are spontaneously fermented before consumption. Spontaneous fermentation of food by lactic acid bacteria (LAB) leads to acid production in food, thus making these foods unsuitable for spoilage organisms to inhabit. In many developing countries, fermented foods serve as major dietary constituents; therefore, they contribute to food safety, add value, and improve nutritional quality and digestibility of foods (Holzapfel 2002; Ogunbanwo et al. 2003; Adesulu and Awojobi, 2014).

LAB are a diverse group of non-motile, non-sporulating Gram-positive bacteria, which thrive under microaerophilic to anaerobic conditions. Taxonomy of these genera suggests that they consist of *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus*, and *Weissella* (Stiles and Holzapfel 1997). The occurrence in fermented foods and feeds as well as long-term use as fermentative microorganisms contributes to their overall acceptance as ‘generally recognized as safe’ (GRAS) status for human consumption. These bacteria has been isolated from diverse African fermented cereals foods, such as *ogi* and *kunun zaki* (Oguntoyinbo and Narbad, 2012, 2015), *koko* (Lei and Jakobsen, 2004), *kenkey* (Halm et al. 1993), *togwa* (Mugula et al. 2003), and *ben-saalga* (Tou et al. 2006). They are associated with the fermentation of many Nigerian indigenous fermented foods such as cassava (to produce *gari*, *lafun,* and *fufu*), maize, and sorghum (to produce *ogi*) (Lartey et al. 1999; Nnam 2000; Sanni et al. 2002; Banwo et al. 2012; Oguntoyinbo and Narbad, 2012, 2015; Adesulu-Dahunsi et al. 2017a). LAB are known to play important roles during the fermentation of these foods (Tamang 2010) and are beneficial microorganisms, which are commonly obtained from various fermented food products globally (Tamang et al. 2012; LeBlanc et al. 2013; Adesulu-Dahunsi et al. 2017a, 2017b). *Lactobacillus plantarum* is the major microorganism involved in the fermentation and nutritional component of *ogi*, fermented cereal gruel (Akinrele 1970; Odunfa 1988). Olukoya et al. (1993) reported the development of an *ogi* product (*dogik*) having therapeutic properties based on its ability to control diarrhea among infants. *Pediococcus* species and *L. fermentum* are associated with the early stages of the fermentation of *ogi* (Banwo et al. 2012, 2013b). Teniola et al. (2005) reported that *Lactobacillus* species is the predominant microorganisms in the fermentation of *ogi-baba*, and it has some beneficial value associated with it. Fermented cassava mash for the production of *gari* had *Lactobacillus* species as the predominant species, especially *L. plantarum, L. fermentum, L. delbrueckii*, and *L. manihotivorans* as well as other gram-positive bacteria (Kostinek et al. 2005; Banwo et al. 2012, 2013a, 2013b). Amoa-Awua et al. (1996) reported that among LAB from *agbelima*, a fermented cassava product from West Africa, *L. plantarum* strains had the highest number, while *Leuconostoc mesenteroides* and *L. brevis* the frequencies of 15% and 16% respectively. Oyewole and Odunfa (1990), who studied the succession of LAB species, observed that *L. plantarum* was predominant after 3 days of fermentation in fermenting cassava. *Lactobacillus plantarum, L. fermentum*, and *Leuconostoc* species occurred in the diversity of LAB from fermented cassava in the production of *gari* (Kostinek et al. 2005). According to Figueroa et al. (1995), the two different LAB groups, which occur in succession, are involved in the fermentation of cassava for *gari* production is mainly *Leuconostoc* and *Lactobacillus* species.

Lactobacillus species, especially *L. plantarum* and *L. fermentum*, were the predominant LAB isolated from *wara*, an African soft cheese (Banwo et al. 2013a).

Banwo et al. (2012) reported that the predominant LAB strains isolated from the fermentation of natural milk for the production of *nunu*, fermented milk curd, were facultative heterofermentative strains of *Lactobacillus* group, tetrad-forming cocci of the *Pediococcus* group and homofermentative cocci, which belong to the *Enterococcus* group. Odunfa (1985) also reported that various LAB strains are involved in the fermentation of Fulani milk *nono* and butter *man shanu*. Akabanda et al. (2013) reported the presence of LAB species: *Lactobacillus helveticus, Enterococcus faecium, Enterococcus italicus, Weissella confusa*, and a presumably novel *Lactococcus* spp. from *nunu*. *Fura* is a spontaneously fermented millet-based dough mixed with spices. It is produced mainly in Nigeria, Burkina Faso, and Ghana in West Africa (Owusu-Kwarteng et al. 2012). The initial stages of the fermentation of *fura* is characterized by co-dominance of homo- and heterofermentative species of *Pediococcus acidilactici, Weissella confusa, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus salivarius*, and *Lactobacillus paraplantarum* while *L. fermentum* was predominant at the end of the fermentation (Owusu-Kwarteng et al. 2012).

2.0.3 Brief Ecology of Yeasts in Indigenous Fermented Foods and Beverages of Africa

Yeasts are predominantly unicellular fungi, which exist throughout nature and are employed in baking and brewing, and currently they are the vital microorganisms used in many commercially important sectors in the production of foods, beverages, pharmaceuticals, and industrial enzymes (Perricone et al. 2014). Involvement of yeasts in numerous indigenous African fermented foods and beverages has been reported (Hounhouigan et al. 1993a, 1993b; Oyewole 2001; Jespersen et al. 2005; Omemu et al. 2007; Greppi et al. 2013b; Ogunremi et al. 2015, 2017). Yeasts play a major role in the natural fermentation of many indigenous food products. They have contributed to the flavor enhancement and general acceptability of fermented cereal gruels (Akinrele 1970; Ogunremi et al. 2015). They are capable of improving the nutritional status of foods (Greppi et al. 2013b) and are reported to have some probioticpotentials (Pedersen et al. 2012; Ogunremi et al. 2015) that help improve human health. The yeasts strains reported to be associated with some African fermented food products are *Saccharomyces cerevisiae, Candida tropicalis, Candida inconspicua, Candida magnolia, Candida humilis, Torulaspora delbrueckii, Kloeckera apiculata, Pichia anomala, Schizosaccharomyces pombe*, and *Kluyveromyces africanus* (Sanni and Lonner 1993; Blandino et al. 2003; Glover et al. 2005; Lyumugabe et al. 2010). The most predominant yeast species reported in indigenous African fermented foods and beverages is *Saccharomyces cerevisiae* (Jespersen 2003).

The two traditional cereal-based fermented foods from Benin, West Africa, are *mawè* and *tchoukoutou* (Hounhouigan et al. 1993a, 1993b; Greppi et al. 2013b). *Mawè* is dehulled maize that is fermented into dough and is used to prepare many dishes such as *agidi* or *eko*, steam-cooked bread (*ablo*), and porridge (*koko, aklui, akluiyonou*). *Tchoukoutou* is majorly opaque sorghum beer, which has sour taste and relatively low alcohol content, ranging from 2–3% v/v, which makes it a valued beverage (Hounhouigan et al. 1993a, 1993d; Greppi et al. 2013b). The microorganisms involved with the fermentation of these beverages are LAB and yeasts. The yeast dynamics in *mawè* are predominantly *Candida krusei* followed by *Candida glabrata* and *Kluyveromyces marxianus*, while in *tchoukoutou Saccharomyces cerevisiae* is the predominant yeast species. Palm wine is an essential beverage in Africa, which is consumed at ceremonies or as a refreshing drink. This beverage is obtained from tapping palm trees such as *Elaeis guineensis*, *Raphia hookeri, Phoenix dactylifera*, and *Cocos nucifera.* The predominant yeast reported in the fermentation of palm wine is *Saccharomyces cerevisiae*, but other yeasts such as *Schizosaccharomyces pombe, Kodamaea ohmeri, Hanseniaspora occidentalis, Candida tropicalis, Kloeckera apiculata*, and *Pichia ohmeri* are also associated with the beverage (Amoa-Awua *et al.* 2007). *Burukutu* is a popular brown-colored alcoholic beverage brewed from sorghum (*Sorghum bicolor*), which is consumed in the Northern Guinea Savanna region of Nigeria, Benin Republic, and Ghana (Iwuoha and Eke, 1996; Odunfa and Oyewole, 1998). The process involves malting, mashing, fermentation, and maturation as described by Faparusi et al. (1973). Fully matured *burukutu* is characterized with vinegary taste and odor. The dominant microorganisms during fermentation is yeast, mainly *Candida* spp., *Saccharomyces cerevisiae*, and *S. chavelieri* and bacteria *Leuconostoc mesenteroides, Lactobacillus* spp., and *Acetobacter* spp. have been reported at the end of maturation (Faparusi et al. 1973; Ogunremi et al. 2015). *Ogi* is a cereal gruel obtained by fermentation of maize, sorghum, and millet (Akinrele 1970; Banwo et al. 2012, 2013b); *gowé* is a malted and fermented maize or sorghum-based food (Vieira-Dalodé et al. 2007; Greppi et al. 2013b). *Kenkey* is a maize-dough produced from whole maize in Ghana, which is similar to *mawè*. Based on the accessible information on the fermentation of these foods, LAB and yeasts are synergistically involved with these products (Akinrele 1970; Hounhouigan et al. 1993b, c; Teniola and Odunfa, 2001; Omemu et al. 2007; Vieira-Dalodé et al. 2007; Oguntoyinbo et al. 2011; Ogunremi et al. 2015; Adesulu-Dahunsi et al. 2018). *Candida krusei* was the predominant yeast species, while *Clavispora lusitaniae* and *Saccharomyces cerevisiae* were detected at lower incidence in the fermentation of *ogi* and *mawè*. The yeast species involved in *tchoukoutou* samples showed less isolates of *C. krusei* but higher levels of *Cl. lusitaniae* and *S. cerevisiae* and a presence of *Candida rugosa*. *Gowé* samples varied from the others with the presence of *Candida tropicalis* and *Kluyveromyces marxianus* occurring in similar levels as *C. krusei* (Greppi et al. 2013a). In the fermentation of *nunu*, a spontaneously fermented yoghurt-like product made in Ghana, Nigeria, and other parts of West Africa, Akabanda et al. (2013) reported the dominance of *Pichia kudriavzevii* and *S. cerevisiae*, while the others are *Candida parapsilosis, Candida rugosa, Candida tropicalis,* and *Galactomyces geotrichum*. The presence of the yeasts species *Candida tropicalis, Candida albicans, Clavispora lusitaniae*, and *Saccharomyces paradoxus* from *akamu* (also known as *ogi* in some regions of Nigeria and West Africa) was reported by Obinna-Echem et al. (2014). Ogunremi et al. (2015) isolated *Pichia kluyveri, P. kudriavzevii, Issatchenkia orientalis*, and *Candida tropicalis* from Nigerian cereal-based fermented foods, which possessed probiotic potentials. Aghemwenhio et al. (2017) reported the presence of four yeast species using phenotypic methods, namely *Saccharomyces cerevisiae, Candida krusei, C. parapsilosis*, and *C. tropicalis* from retting cassava. The study of *pito* production sites in different parts of Ghana for yeast biodiversity revealed that 99% of the yeast isolates showed macro- and micromorphological characteristics typical of *Saccharomyces cerevisiae* (Glover et al. 2005). *S. cerevisiae* is the dominant microorganism from the microbiological analysis of Rwandese traditional beer *ikigage* (Lyumugabe et al. 2010).

There are varieties of autochthonous yeast species involved in the traditional fermentation of foods and beverages in Africa. This suggests that the yeast population responsible for the fermentation of traditional fermented foods and beverages in Africa may vary depending on area, raw materials, processing conditions, occurrence of other microorganisms, and local deviations in the production processes (Sanni and Lonner, 1993; Ogunremi et al. 2017).

3.0 Molecular Techniques for the Identification of Microorganisms in African Indigenous Fermented Foods

3.0.1 Genotypic Identification of *Bacillus* Species and Associated Microorganisms from Fermented Vegetable Condiments of Africa

The vegetable seeds used for the production of condiments differ extensively from one region of Africa to another (Achi 2005; Adewumi et al. 2013). However, the production process is by alkaline fermentation of spontaneous solid substrate with an increase in pH. This results in hydrolysis of the proteins into peptides, amino acids, and ammonia, which favors the predominance of *Bacillus* spp. as the fermenting organisms (Ouoba et al. 2004; Achi 2005; Adewumi et al. 2013). The identification of the microorganisms involved in the fermentation of vegetable condiments in earlier studies had been based on phenotypic characterization using biochemical tests only; the biochemical tests are poorly discriminatory, non-reproducible, and often laborious. This method of characterization is often not reliable and lack accurate detection of the microbial diversity present in the fermenting vegetable proteins (Achi 2005; Adewumi et al. 2013).

Conventional methods of characterization of *Bacillus* species does not always delineate among different species in the genus because of phenotypic relatedness (Oguntoyinbo et al. 2010). Even though, *Bacillus subtilis* has been reported as the most essential species involved in fermentation of vegetable condiments (Odunfa 1981; Sanni and Ogbonna, 1991; Omafuvbe et al. 2003; Ouoba et al. 2004; Parkouda et al. 2009; Oguntoyinbo et al. 2007, 2010), most of the strains studied in diversity investigations were only phenotypically identified (Odunfa 1981; Sanni and Onilude, 1999; Omafuvbe et al. 2003). The *B. subtilis* group comprises strains of *B. subtilis, B. vallismortis, B. mojavensis, B. atrophaeus, B. amyloliquefaciens, B. licheniformis, B. sonorensis, B. firmus, B. lentus, B. megaterium*, and *B. pumilus*, which are phenotypically related and are often difficult to differentiate (Oguntoyinbo et al. 2010). Difficulties in phenotypic characterization are associated with the *Bacillus cereus* group, which comprises *B. cereus, B. mycoides, B. weihenstephanensis, B. pseudomycoides, B. anthracis*, and *B. thuringiensis* (Oguntoyinbo et al. 2010).

Culture-dependent techniques use DNA typing procedures for characterization and molecular identification of *Bacillus* species obtained from vegetable condiments and other traditional fermented foods. These techniques include the amplification of PCR of the 16S-23S rDNA internal transcribed spacer (ITS-PCR) for diversity of species, restriction fragment length polymorphism of the ITS-PCR (ITS-PCR-RFLP) for speciation of species and strains, and pulsed field gel electrophoresis (PFGE) for differentiation of strains and sequencing of 16S rDNA for description of phylogenetic relationships (Ouoba et al. 2004). The combination of PCR of the internal transcribed spacer and repetitive element PCR was employed to distinguish among bacilli from *bikalga*, a vegetable condiment from *Hibiscus sabdariffa* (Ouoba et al. 2008; Oguntoyinbo et al. 2010).

Culture-independent microbial techniques, such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE), have been employed in the microbial profiling complex of bacterial community, dynamics, and evolutionary population of *Bacillus* spp. in fermented vegetable condiments (Cocolin et al. 2007; Adewumi et al. 2013). PCR-DGGE has been effectively employed to study the community and dynamics of microbes involved in fermented vegetable protein seeds such as *doenjang* and *meju* from Asia and *iru* from Nigeria, West Africa (Kim et al. 2009; Lee et al. 2010; Adewumi et al. 2013). The differences among treatments in the *Bacillus*-related community had distinct classification employing PCR-DGGE (Parkouda et al. 2015). The related sequences were associated with *Bacillus mycoides, B. pumilus, B. megaterium,* and *B. thuringiensis*, while related taxa such as *Paenibacillus* were obtained (Parkouda et al. 2015). PCR-DGGE sequencing identified *B. subtilis* as the major bacterial species associated with the fermentation of *iru*, which is in agreement with the result of culture-based PCR analysis (Adewumi et al. 2013).

Culture-dependent analysis and 16S rRNA gene sequencing exhibited *iru* to comprise *Bacillus* species, which possessed clonal relationship and identified as *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, and *Brevibacillus formosus* in order of occurrence. The combination of ARDRA, ITS-PCR, ITS-PCR-RFLP, and RAPD-PCR showed a high degree of diversity among the *Bacillus* isolates and identified the dominant species to be *B. subtilis* and *B. amyloliquefaciens* (Adewumi et al. 2013). The authors distinctly indicated that subtyping of autochthonous species of bacilli with distinct clonal relationship and genetically identified predominant groups is in agreement with previous reports in W. Africa and India during fermentation of similar vegetable proteins (Ouoba et al. 2004; Jeyaram et al. 2008; Kim et al. 2010; Oguntoyinbo et al. 2010; Parkouda et al. 2010). Oguntoyinbo et al. (2010) reported that a polyphasic taxonomic approach to characterize the dominant *Bacillus* isolates in Nigerian *okpehe* fermentation was very vital and important. The authors asserted that ARDRA genotyping method with the enzymes *Hha*I, *Hinf*I, *and Sau3A*I was not successful in the differentiation of the *Bacillus* species, but was able to delineate the *B. cereus* group from other *Bacillus* species. The challenges of speciation were achieved with the combination of RAPD-PCR and ARDRA genotyping methods. The authors reported that polyphasic taxonomic approach was successful in separating the dominant *Bacillus* species in *okpehe* samples into *B. subtilis, B. amyloliquefaciens, B. cereus, and B. licheniformis* in decreasing order of occurrence.

Ouoba et al. (2004) reported that ITS-PCR and ITS-PCR-RFLP were suitable for typing of *Bacillus* isolates from *soumbala* at species level, with a minor variation at strain level for one isolate of *B. subtilis* and the two of *B. pumilus.* The ITS-PCR was observed to be suitable for typing *B. subtilis* and *Bacillus* spp. from another origin only to species level (Shangkuan et al. 2000; Ouoba et al. 2003; Ouoba et al. 2004). The suitability of typing *Bacillus* spp. at strain level by the digestion of the PCR product with restriction enzymes has also been reported to be effective (Shangkuan et al. 2000; Ouoba et al. 2004). The most discriminative method for the identification of *Bacillus* spp. from *soumbala* was PFGE, which was suitable for the typing of *B. subtilis, B. licheniformis*, and *B. cereus* at strain level with the use of *Sfi*I and *Sma*I as restriction enzymes (Ouoba et al. 2004). Ouoba et al. (2004) reported that ITS-PCR and ITS-PCR-RFLP (using one restriction enzyme) are simple methods, which are equally discriminatory for the typing of *Bacillus* spp. from vegetable condiments. Earlier studies using traditional culture-dependent methods and biochemical characterization reported that the predominance of *B. subtilis* during fermentation of*iru* and other vegetable condiments (Odunfa and Oyewole, 1986; Ouoba et al. 2004; Oguntoyinbo et al. 2010; Adewumi et al. 2013).

3.0.2 Genotypic Identification of Lactic Acid Bacteria Isolated from Indigenous Fermented Foods of Africa

The use of genotypic methods of classification and differentiation is very imperative for proper identification of microorganisms from traditional fermented foods (Banwo et al. 2012). The characterization and identification of LAB could not depend on the phenotypic methods only. Genotypic methods are employed to dissuade the inconsistency, ambiguity, and misidentifications often associated with phenotypic means of characterization of microorganisms, especially LAB strains (Kostinek et al. 2005; Banwo et al. 2012). Therefore, reliable identification of LAB at the subspecies and strain level is of great importance to understand the activities (Banwo et al. 2012; Owusu-Kwarteng et al. 2012; Akabanda et al. 2013). The use of molecular tools such as random amplified polymorphic DNA (RAPD-PCR), repetitive extragenic palindromic of the DNA elements (rep-PCR) and 16S rDNA gene sequencing has proved useful in the resolution of the identities of the LAB strains (Gevers et al. 2001; Kostinek et al. 2005; Banwo et al. 2012; Owusu-Kwarteng et al. 2012; Akabanda et al. 2013; Sanni et al. 2013).

Molecular methods employing PCR and sequencing of the 16SrRNA gene extensively used for LAB identification allows for the diversities among strains of the same species. The PCR based fingerprinting approaches has been reported by researchers as consistent and reliable tools for proper identification of these bacteria to species and strain level (Banwo et al. 2012; Sanni et al.

2013; Adesulu-Dahunsi et al. 2017a, 2017b). Determination of the intra-species strain diversity is important in investigating the functionality of the strains (Leroy and DeVuyst, 2004; Sanni et al. 2013). Several studies have reported the diversity within LAB using different PCR-based methods such as RAPD-PCR, PFGE, multilocus sequence typing (MLST) and sequencing of 16S rDNA (Banwo et al. 2012; Sanni et al. 2013; Adesulu-Dahunsi et al. 2017a). Randomly amplified polymorphic DNA (RAPD-PCR) has been applied to species and strains differentiation of several LAB. In most cases, RAPD requires more than one primer to characterize strains. Since this technique uses non-specific amplification conditions, such as low annealing temperature, the reproducibility of DNA fingerprints is usually low. Pulsed-field gel electrophoresis is one of the most versatile molecular tools for characterizing to strain level, and it involves the digestion of the genomic DNA with restriction enzymes. The RAPD-PCR and PFGE techniques ensure the differentiation of LAB strains from diverse sources and geographical origins (Chen et al. 2012; Adesulu-Dahunsi et al. 2017a). Banwo et al. (2012) reported that RAPD-PCR with primer M13 possessed a specific level of heterogeneity among LAB strains from isolated from different fermented foods. The use of RAPD-PCR method with different oligonucleotide for *Lactobacillus* strain differentiation has been documented (Banwo et al. 2012; Sanni et al. 2013). Samarzija et al. (2001) asserted that the RAPD method is a useful and reliable method for the genetic diversity among LAB and discriminates *Lactococcus lactis* subsp. *cremoris* at strain level. Gevers et al. (2001) affirmed that repetitive extragenic palindromic (rep-PCR) typing using the primer (GTG)5 is a powerful technique for accurate identification of LAB strains. Banwo et al. (2012) observed that rep-PCR had a good discriminating power to place all the strains in their study into their respective taxonomic groups with high identification scores. Other molecular methods such as ribotyping, amplified ribosomal DNA restriction analysis (ARDRA), internal transcribed spacer (ITS-PCR) and amplified fragment length polymorphism (AFLP) have been established in the identification of LAB (Jeyaram et al. 2010; Adesulu-Dahunsi et al. 2017b). Among these techniques, amplified ribosomal DNA restriction analysis has been employed as a consistent molecular technique for evidently discriminating closely related species of *Bacillus* (Oguntoyinbo et al. 2010; Adewumi et al. 2014). The 16S and 23S rDNA internal transcribed spacer typing method varies in the sequences from one species to another thus providing greater genetic variation (Tilsala-Timisjarvi and Alatossava, 1997; Adesulu-Dahunsi et al. 2017b).

The applicability of ARDRA profile using *Hae*III restriction endonuclease method for rapid identification of *L. plantarum* species was previously confirmed (Adesulu-Dahunsi et al. 2017a). Adesulu-Dahunsi et al. (2017a) reported that differentiation of *L. plantarum* achieved by ITS-RFLP, RAPD, and PFGE analysis allowed identification to strain levels. Differences was not observed in the restriction patterns among the strains of *L. plantarum* with the amplification of the 16S-23S rDNA ITS-PCR, but diversity was seen in the ITS-RFLP pattern among the *L. plantarum* strains digested with *Hae*III restriction enzyme (Adesulu-Dahunsi et al. 2017a). The digestion of ITS-RFLP-PCR with *Hae*III restriction enzyme profile showed intra-species relationship among the strains of *L. plantarum*. Jeyaram et al. (2011) reported a high intra-species variation among the *Bacillus subtilis* species using ITS-RFLP corroborated this finding. Pulsed-field gel electrophoresis (PFGE) has been extensively used as a molecular tool for the analysis of the diversity of LAB from various food sources and geographical regions to subspecies and strain level using two restriction enzymes *Apa*I and *Sfi*I (Anna et al. 2008; Adesulu-Dahunsi et al. 2017b). Several researchers have reported that PFGE can differentiate strains among LAB species than any other molecular typing methods (Pepe et al. 2004; Adesulu-Dahunsi et al. 2017a).

LAB isolates and reference strains were taxonomically identified with ARDRA-based technique using three different restriction endonuclease enzymes *Hae*III, *Hinf*I, and *Rsa*I. This technique differentiated LAB isolates with the digestion of restriction enzyme, *Hae*III. The ARDRA technique allowed for the discrimination of *Lactobacillus plantarum, Pediococcus pentosaceus*, and *Weissella confusa* with the use of only one restriction enzyme, *Hae*III, with distinct profiles obtained for each species. This is evident that ARDRA can successfully differentiate LAB into species levels using suitable restriction endonuclease(s) (Adesulu-Dahunsi et al. 2017a, 2017b). The ITS region is distinct in characterization and identification of bacteria to species level

(Mohammed et al. 2011; Adesulu-Dahunsi et al. 2017a). PCR amplification of the 16S-23S rRNA gene internal transcribed spacer (ITS-PCR) sustained the interspecies differentiation produced by ARDRA. The length variation in ITS region is principally due to the type and number of transport RNA genes combined, while the differences in the band intensities is due to the number of ribosomal RNA operons (Moreira et al. 2005; Adesulu-Dahunsi et al. 2017b). The 16S rDNA gene sequencing of the LAB strains align with the ARDRA and ITS-PCR clusters and rep-PCR technique as reported by Banwo et al. (2012). The ARDRA and ITS-PCR are inexpensive, rapid, consistent, and reproducible in comparison with other techniques (Oguntoyinbo et al. 2010; Adesulu-Dahunsi et al. 2017a, b). The identification of LAB from traditional fermented foods employing molecular techniques reduces labor, time, and enhances rapid and accurate identification. These molecular techniques favorably complement the phenotypic method of LAB species identification. Analyses resulting from more than one molecular method are important for the typing of LAB because of the high degree of genetic variation among them.

3.0.3 Genotypic Identification of Yeasts Isolated from Indigenous Fermented Foods of Africa

Understanding the dynamics in yeasts classification is very imperative to their roles in the effective fermentation process, which influences the quality of the final product (Greppi et al. 2013a, 2013b). In the study of yeast dynamics and populations, current taxonomic databases, culture-independent techniques such as PCR denaturing gradient gel electrophoresis (DGGE) and PCR temporal temperature gradient gel electrophoresis (PCR-TTGE) are needed. These techniques have been extensively employed for investigating microbial dynamics in complex food matrices (Ramos et al. 2010; Greppi et al. 2013a) and in the exploration of yeast diversities in foods (Cocolin et al. 2002; Chang et al. 2008; Greppi et al. 2013b). However, amplification of a particular region of the ribosomal RNA aimed at identification of a specific bacterial community may cause a constraint in understanding the broader knowledge of the entire population (Greppi et al. 2013a, 2013b). Molecular techniques used to identify and discriminate yeast species and strains are amplification of PCR using specific primers, which include restriction fragment length polymorphism (RFLP) analysis of 5.8 S-ITS region (Bautista-Gallego et al. 2013) and sequencing of the D1/D2 domains of 26S rDNA gene (Bautista-Gallego et al. 2013; Ogunremi et al. 2015). In addition, amplification of the ITS1-5.8S rDNA-ITS2/16S-23S rDNA ITS regions and rep-PCR analysis using the primer (GTG)5 were employed by some researchers for the identification of predominant yeast species isolated from traditional fermented foods and beverages (Akabanda et al. 2013; Greppi et al. 2013a; Ouoba et al. 2013).

Yeast diversity investigated by rep-PCR typing is receiving a notable attention in the area of food fermentation because it is useful in understanding the dynamics of these microorganisms during fermentation, and it assists in identifying if a particular culture inoculated as starter is able to dominate the fermentation (Cocolin et al. 2011; Greppi et al. 2013b). This molecular technique revealed a succession of biotypes of *C. glabrata* during the fermentation of *mawè* (Greppi et al. 2013a). In previous study, Greppi et al. (2013a) reported a variety of biotypes of *C. krusei* in *mawè* from different locations in Benin. The composition and microbiology of the raw materials from the several production locations and operators influence the diversities of the microbial communities obtained from their fermentation (Jespersen et al. 2005; Greppi et al. 2013a, 2013b).

Akabanda et al. (2013) reported the grouping of yeasts isolates from *nunu* using (GTG)5-based rep-PCR fingerprinting and representative isolates from each group were identified by sequencing of the D1/D2-region of the 26S rRNA gene. Ouoba et al. (2013) reported significant genotypic diversity among the species of *Candida tropicalis* obtained from the palm sap of *bandji,* which displayed three subgroups for five isolates using rep-PCR. The authors observed limited genetic diversity for *S. cerevisiae* and *Trichosporon asahii* and no diversity for *Arthroascus fermentans* and *Issatchenkia orientalis* using rep-PCR. Conversely, these species displayed phenotypic diversity in the sugar fermentation, while *C. tropicalis* demonstrated the greatest diversity. This study indicated that multi-yeast starters are associated with palm sap fermentation of *bandji*, whether at the species or subspecies/strain level (Ouoba et al. 2013).

The DGGE technique combined with cultural methods has been useful to study the yeast ecology of *ogi, gowe, mawè,* and *tchoukoutou* final products from Benin (Greppi et al. 2013a, 2013b). The detection limit of DGGE analysis for yeasts is about 10^3 cfu/g or ml (Cocolin et al. 2001; Greppi et al. 2013a, b), and if the populations in the food samples is small, it may be difficult to be detected as DGGE bands (Greppi et al. 2013a). The study showed that *Kluyveromyces marxianus, C. glabrata,* and *C. krusei* as the species present and metabolically active during *mawè* fermentation. Identified during the whole fermentation from the total DNA and RNA was *K. marxianus*, while *C. glabrata* and *C. krusei* were identified at both DNA and RNA levels signifying their active contribution to the fermentation (Greppi et al. 2013a, 2013b). The authors confirmed that the cultural data conforms to the molecular technique with the exception of the absence of *S. cerevisiae*, identified in high percentage at the last stages of the fermentation. They asserted that it could be due to PCR bias in the food matrices where several yeast species are present, thereby interfering with the specific binding of the primers to other species. The presence of a DGGE band obtained from first sampling location of *mawè* samples close to *Zea mays* in the GenBank database indicates the limitations of the set of primers used in this study (Greppi et al. 2013a, 2013b).

4.0 Future Trends for Molecular Techniques in Fermented Food Microbiology in Africa

There are increasing numbers of traditional fermented foods that possess health benefits. Consumers in Africa are getting educated and aware of the positive role of fermented food on health promotion and general well-being, which calls for an important prospect in the advancement of fermented food research. This will involve the unified systems approach, which will develop our knowledge of how certain microorganisms from the different regions of Africa ferment foods, which confers functionality on the foods for the diverse populations (Borresen et al. 2012). This chapter emphasized microorganisms involved in fermented foods and beverages and how different methods of taxonomic classification aided in the identification. There is a call for the use of the polyphasic approach for the identification of microorganisms involving phenotypic and several genotypic methods, which will aid the investigation of their functionality as starter culture(s) in traditional fermented foods in Nigeria and sub-Saharan Africa (Banwo et al. 2012; Sanni et al. 2013; Adesulu-Dahunsi et al. 2017b). Strain-level typing of microorganisms is vital for the selection of the best strains for biotechnological, industrial, pharmaceutical, and environmental studies. Different genomic typing techniques has been discussed in this chapter to determine phylogenetic relationship and diversity among microorganisms isolated from indigenous African fermented foods. Molecular fingerprinting such as rep-PCR, RAPD-PCR, PFGE, amplification of the 16S-23S rDNA internal transcribed spacer (ITS) PCR, and ARDRA techniques are useful for delineation of microorganisms isolated from different fermented food products. The microorganisms exhibited varied profile, which confirmed vast diversity of microbial communities from different indigenous African fermented foods (Oguntoyinbo et al. 2010; Banwo et al. 2012; Akabanda et al. 2013; Ouoba et al. 2013; Sanni et al. 2013; Adesulu-Dahunsi et al. 2017a, 2017b). These techniques used singly or in combination aid in better reliability, rapid identification, and differentiation of microorganisms obtained from indigenous fermented foods (Adesulu-Dahunsi et al. 2017a, 2017b). The combination of culture-dependent and culture-independent techniques is paramount to outline microorganisms, examine the dynamics and diversities involved in the fermentation of indigenous fermented foods (Oguntoyinbo et al. 2011; Adewumi et al. 2013). These features will complement the need for a thorough understanding of the dynamics of the microorganisms isolated from indigenous fermented foods to understand the insight of their activities and the qualities they confer on fermented food products in terms of appearance, taste, nutrition, and safety.

African researchers should collaborate in the preservation of microorganisms isolated, adequately characterized, and identified from indigenous fermented foods. The genome of the strains with distinct qualities can undergo complete sequencing for continuous use in research. This can be achieved by developing a culture collection center for these microorganisms, which can be typed strains based on the features they possess so that they can be accessed for research purposes just like the American Type Culture Collection (ATCC), German Collection of Microorganisms and Cell Cultures (DSMZ), Japanese Culture of Microorganisms (JCM), and China General Microbiological Culture Collection (CGMCC). These organizations acquire, validate, preserve, propagate, and distribute microorganisms with their information for scientific research purposes. They are all non-profit organizations that serve and support the scientific community with a diverse collection of microorganisms.

In conclusion, polyphasic taxonomic techniques were useful in strain identification and comprehensive understanding of the diversities of microorganisms isolated from different indigenous fermented foods in Africa (Oguntoyinbo et al. 2010; Banwo et al. 2012; Adewumi et al. 2013; Akabanda et al. 2013; Ouoba et al. 2013; Sanni et al. 2013; Adesulu-Dahunsi et al. 2017a, b).

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Section 8

Therapeutics and Fermented Foods

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23

Therapeutic Uses of Fermented Food Products

Nirmaladevi, R.
Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India

1 Introduction

Fermented food products constitute a significant part of the diet in most of the developing and developed nations. Fermented foods contribute to about one-third of the diet worldwide. There is a renewed interest in traditional fermented foods in recent times, mainly due to the apparent health benefits of fermented foods both as vehicles of probiotic organisms and health-promoting metabolites. Fermented foods are currently being promoted to prevent or cure an array of diseases ranging from obesity to cancer. Fermentation process is a conversion of complex organic substances, mostly carbohydrates, into simpler compounds by the action of microorganisms such as bacteria, yeasts, or molds. From the chemical perspective, fermentation consists of a series of reactions catalyzed by the enzymes in the living cells. Fermentation is one of the oldest methods of food processing and preservation, which increases the nutritive value of food, making it easily digestible and tastier with enhanced aroma, and also prevents its spoilage thereby increasing the shelf life of the product.

Food materials are fermented since ancient times as a food processing and preservation technique by employing microorganisms such as bacteria and yeast which use nutrients in the food as energy source. These microorganisms then produce organic acids and other compounds beneficial for health. Fermentation is a cheap and energy-efficient process. Fermentation improves the shelf life and taste of the food products. It also gives flavor and aroma to the food products. Many studies on fermented food products revealed that they possess antioxidant, antiatherosclerotic, hypolipidaemic, immunoregulatory, antimicrobial, antihypertensive, and antithrombotic properties, proteolytic activity, and prevent diarrhea and lactose intolerance.

Commercially available drugs used during the treatment of diseases cause more side effects that include nausea, vomitting, skin irritation, stomach upset, allergy, dry mouth and they are more expensive as well. But the limited consumption of fermented food products doesn't produce any side effects and they are cost effective and nutrient rich.

This review is a compilation of therapeutic uses of various fermented food products including fermented dairy, soy, and sea food products and also some fermented beverages.

2 Fermented Dairy Products

Milk products prepared by fermentation with lactic acid bacteria or combination of yeast and lactic acid bacteria are called fermented dairy products. Some species of lactic acid bacteria present in the gut of healthy individuals are introduced through the consumption of fermented milk products that contain *Lactobacillus* species. They give nutritional and health benefits to the

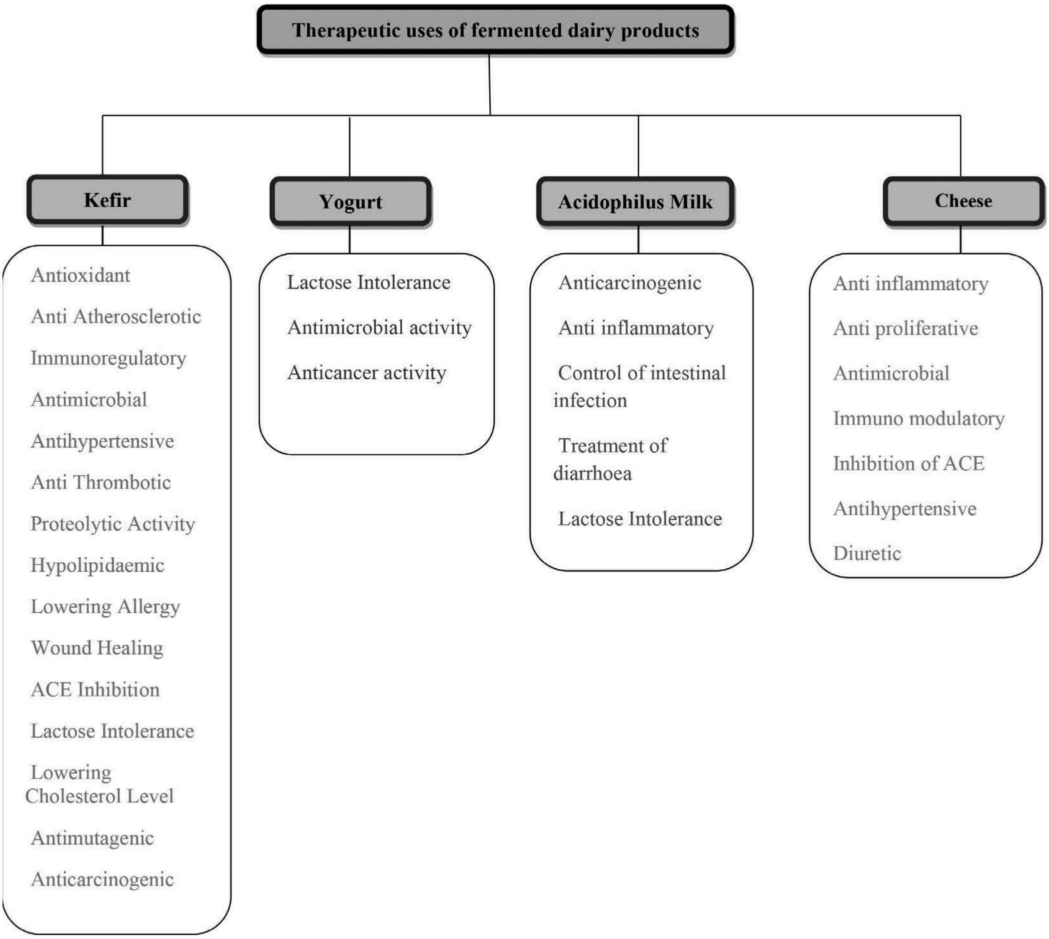

FIGURE 23.1 Therapeutic uses of fermented dairy products.

consumer. Lactic acid bacteria produce various enzymes and vitamins and are responsible for the proper metabolism of the host.

There are two kinds of microorganisms categorized based on the optimum temperature range it works, that are employed for the production of fermented dairy products. Lactic acid bacteria incubated at 35°C are called thermophilic starters, and those incubated at 20°–30°C are called mesophilic starters. The combination of selected lactic acid bacteria and their strains produces different fermented products (Chandan, 2014).

Fermented dairy products including kefir, acidophilus milk, yogurt, and cheese produce numerous health benefits that are discussed Fig 23.1.

2.1 Kefir

Kefir is a traditional fermented dairy product produced using kefir grains. It is obtained by the action of acidic bacteria and yeast (Figure 23.2). Recent studies suggest that kefir can be used for the treatment of several diseases including inflammatory and cardiovascular diseases. Kefir is also found to possess biological activities such as antioxidant, antiatherosclerotic, hypolipidaemic, immunoregulatory, antimicrobial, antihypertensive, antithrombotic, and proteolytic (Santanna

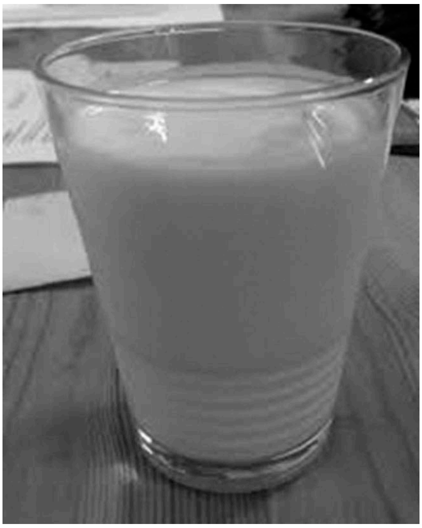

FIGURE 23.2 Kefir.

et al., 2016). In addition, kefir is also effective against some clinical conditions such as digestive/gastrointestinal disorders, lowering allergy, wound healing and ACE inhibition, preventing lactose intolerance (Erdogan *et al.,* 2018)

Kefir, the fermented dairy beverage from the Caucasus, is claimed to reduce lactose intolerance symptoms, stimulate the immune system, lower the cholesterol level, and have antimutagenic and anticancer properties. Several studies on the anticarcinogenic activity of kefir indicate that it is effective against several types of cancer including lung cancer (inhibition of pulmonary metastasis), mammary cancer, sarcoma (reduces proliferation and apoptosis), and leukemia (inhibits proliferation).

2.2 Yogurt

Yogurt, a semi-solid fermented dairy product is produced from pasteurized milk by fermentation with thermophilic cultures, including *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. and *Bulgaricus* as starter cultures (Figure 23.3). *Streptococcus thermophilus* is responsible for the production of lactic acid which improves milk acidification. The role of *L. delbrueckii sp. and Bulgaricus* is to give aroma and flavor to the yogurt (Innocente *et al.,* 2016). It is found to posses numerous health benefits including lactose intolerance, since fermented foods often contain probiotics, or beneficial microbes,

FIGURE 23.3 Yogurt.

which may lower the risk for certain types of cancer, improve immune function, enhance the availability of certain nutrients, and improve the intestinal health and antimicrobial activity.

a) Lactose intolerance

The inability to digest lactose, a component of milk and some dairy products is called lactose intolerance. Lactose intolerance is due to the deficiency of the enzyme lactase. Consuming fermented milks like yogurt can improve lactose maldigestion. The bacterium present in the yogurt releases β-galactosidase and gets accumulated in the stomach of the lactase-deficient person and acts as a substituent for lactase. When lactobacillus acidophilus colonies are present in the intestine, utilization of lactose is found to be improved, indicating its potential against lactose intolerance. So an enhanced absorption of lactose is seen after consuming yogurt (Litopoulou-Tzanetaki and Tzanetakis, 2014).

b) Antimicrobial Activity

Yogurt starter bacteria *Lactobacillus delbrueckii* sp. *Bulgaricus* produces an antimicrobial agent 'bulgarican,' which is a kind of baceriocin that inhibits pathogenic bacteria. Bulgarican produces a broad spectrum antimicrobial activity against a wide range of microorganisms including *Salmonella*, *Shigella*, *Escherichia coli*, and *Pseudomonas* (Chandan *et al.,* 2017).

c) Anticancer Activity

Cancer is one of the leading causes of death worldwide. Yogurt acts against certain type of cancers such as breast cancer, ovarian cancer and colon cancer. Several studies using animals suggested that lactic acid bacteria present in the fermented milk is responsible for the prevention of cancer initiation or suppression of initiated cancer. The mechanism involved in anticarcinogenic activity includes adsorption of food-borne carcinogens, stimulation of protective enzymes, increase in immune response, reduction of tumor promoters, and so on. Several studies indicated that after consumption of fermented milk containing lactic acid bacteria, there is a notable increase in cytokine production, antibody production, phagocytic activity, T-cell production, etc., thereby preventing the host against cancer (Litopoulou-Tzanetaki and Tzanetakis, 2014).

2.3 Acidophilus Milk

Acidophilus milk is a fermented milk product produced by bacterial fermentation using *Lactobacillus acidophilus.* It is a popular product and consumed more in the United States. Many studies revealed that consumption of fermented milk containing *Lactobacillus acidophilus* produces several health benefits which includes prevention and control of intestinal infection, anticarcinogenic activity, prevention of lactose intolerance, and used in the treatment of diarrhea (Kongo and Malcata, 2016).

a) Control of Serum Cholesterol

Some *L. acidophilus* strains from human origin are found to remove cholesterol from culture medium when they are grown in the presence of bile. The probable mechanism involved might be that, these strains deconjugate bile acids and co-precipitate with cholesterol at optimum pH (<5.5). The loss of bile acids will be compensated by the liver by converting cholesterol into bile acids. Because of this conversion, serum cholesterol level will be lowered which reduces the risk of coronary heart disease (Kießling *et al.,* 2002). But a single strain of *L. acidophilus* will not produce this effect. Selection of specific strains of *L. acidophilus* and combining them together is necessary to attain this beneficial effect (Gilhland, 1989). It is clearly evident that a regular intake of fermented milk containing specific *L. acidophilus* strains will reduce the risk of coronary heart

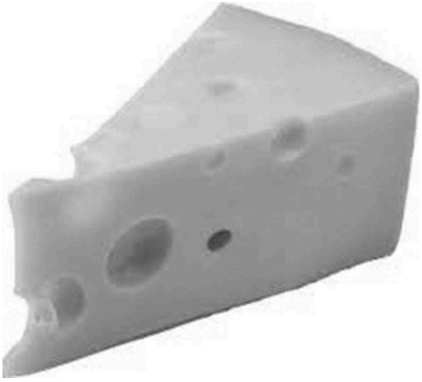

FIGURE 23.4 Cheese.

disease, because even 1% reduction of serum cholesterol level will reduce the risk of coronary heart disease upto 2–3% (Kießling *et al.,* 2002).

2.4 Cheese

There are 400 varieties of cheese consumed throughout the world. Cheese is produced by converting liquid milk into a semisolid form using a coagulating agent or acid (Figure 23.4). Lactic acid bacteria play a major role in the cheese-making process. Most varieties of cheese are produced using mesophilic cultures, but a few varieties need thermophilic starter cultures. Cheese production process involves lowering of pH and addition of salt and it confers a preservative effect. Cheese provides rich nutrition, flavor, and texture to the consumers (Chandan, 2014). Cheese posses numerous health benefits including anti-inflammatory, antioxidant, antiproliferative, antimicrobial (via producing antimicrobial peptides, e.g. bacteriocins) and immunomodulatoty activities. In addition to this, it inhibits angiotensin-converting enzyme thereby preventing cardiovascular diseases, induces hypotension, and acts as diuretic agent (Santiago-López *et al.,* 2018).

3 Fermented Soy Products

Generally soy products are fermented using *Bacillus subtilis* and *Aspergillus* species. Based on the preparation method and type of microbial strain used for the fermentation process, soy products have different names in the market. Fermentation can improve the physicochemical properties and quality of the soy products. Fermented soy bean products are found to posses various biological activities including antioxidant, anti-inflammatory, antihypertensive, antimicrobial, anti-osteoporotic, antidiabetic, and immunostimulant properties. The major and predominant component in these fermented food products is isoflavones which are responsible for these biological activities.

3.1 Tempeh

Tempeh initially originated from Indonesia, but now it is consumed in many developing countries since it is easy to prepare and also cost effective. Tempeh is a fermented soy bean product produced using *Rhizopus* species (Figure 23.5). It is rich in vitamin B_{12} and it is a low-cost protein source, providing essential and non-essential aminoacids. Isoflavones and proteins present in tempeh are responsible for the health benefits of gastrointestinal tract. Since it is rich in protein, tempeh consumption increases muscle mass (Mani and Ming, 2017). Therapeutic uses of tempeh are as follows:

FIGURE 23.5 Tempeh.

a) Antioxidant Activity

Fermented soy products are found to contain large amounts of isoflavones, a phytoestrogen antioxidant (Lin *et al.,* 2006). Dietary intake of soybean isoflavones for 30 days enhances the antioxidant status. A potent antioxidant compound 3-hydroxyanthranilic acid (HAA) is found to be present in tempeh. These compounds are responsible for the antioxidant activity of soy products.

b) Hypocholesterolemia and hypertension

Consumption of Soybean proteins were found to reduce total cholesterol and triglycerides in animals as well as humans (Lin *et al.,* 2004) A recent investigation concludes that continuous intake of acid fermented soy milk for 5 weeks showed a reduction in liver triglycerides and cholesterol and also the gene involved in cholesterol synthesis SREBP-2 is found to be down-regulated (Kobayashi *et al.,* 2012). An animal study using rat model suggested that administration of tempeh for 28 days reduced mean body weight of the experimental animals (Babji *et al.,* 2010). Consumption of Gamma-Aminobutyric Acid (GABA) enriched-tempeh acts as an antihypertensive agent. Also oleic acid and linoleic acid were found in the fermented soy products. These fatty acids inhibit HMG-CoA reductase enzyme which in turn inhibits cholesterol biosynthesis thereby reducing the risk for cardiovascular diseases.

c) Cancer Prevention

Recent studies showed that fermented soy products play a major role in the prevention of cancer. Regular consumption of soy products reduces the risk of hormone-dependent cancers including breast and prostate cancers because of the action of soybean isoflavones such as genistein and daidzein. Soy products influence carcinogenesis at the stages of initiation, promotion, and progression. Genistein exhibits antitumor effect against pancreatic cancer. Genistein present in the soybean is responsible for the inhibition of protease production, cell detachment, and cell invasion and thereby controls human prostate cancer. Colorectal cancer is the most common cancer among human population and the mortality rate is comparatively more than some cancer types. Genistein inhibits cell proliferation and induces apoptosis in colorectal cancer cells (Qin *et al.,* 2016).

3.2 Natto

It is a traditional soy bean product of Japan. Natto is produced from yellow soybeans through fermentation by *B. subtilis.* The major constituents of natto are sugars, amino acids, vitamins, fats, proteins, dietary fibres, and minerals (Figure 23.6). It is found to possess health benefits such as

FIGURE 23.6 Natto.

anticoagulant, fibrinolytic, and blood pressure lowering effects due to the presence of the enzyme nattokinase (Mani and Ming, 2017).

a) Anti-thrombotic activity

Natto contains several biological factors that exhibit anti-thrombotic activity, which include nattokinase, dipicolinic acid, and bacillopeptidase F. Among these, nattokinase plays an important role; it is an extracellular subtilisin like serine protease produced by *B. subtilis* (involved in natto production). This nattokinase dissolves fibrin and exhibits greater fibrinolytic activity (Cao *et al.,* 2018).

b) Bone Density and Osteoporosis

Vitamin K_2 and calcium present in natto are responsible for the improvement of bone health. Several studies revealed that dietary intake of natto lowers the incidence of hip fracture (Kaneki *et al.,* 2001), increases bone strength (Katsuyama *et al.,* 2002, 2004), and enhances bone mineralization density (Ikeda *et al.,* 2006).

3.3 Other Fermented Soy Bean Products

- **Doenjang**

It is a fermented soybean food originated from Korea. It is prepared by soaking and crushing the soy bean. Then the crushed soybean is allowed to be fermented with *Bacillus subtilis, Aspergillus* sp., and *Rhizopus* sp., over a period of 2 to 3 months. It is rich in isoflavones, vitamins, minerals and phytosterols. Therapeutic uses of doejang include prevention of cancer, heart disease, brain tumors and regulation of blood pressure and cholesterol levels and also promote gut health (Jeong *et al.,* 2014).

- **Cheonggukjang**

Cheonggukjang is a traditional Korean soybean paste fermented using *Bacillus subtilis.* It contains ground as well as whole soybeans. In addition to its nutritional value, it possess antioxidant, anti-inflammatory, antihypertensive, neuroprotective, antimicrobial, anti-osteoporotic, antidiabetic, and immunostimulant properties (Cho *et al.,* 2011).

- **Doubanjiang and Tauchu (Tauco)**

Doubanjiang is a traditional fermented soy bean product originated from China through fermentation with *Aspergillus oryzae.* Isoflavones present in *Doubanjiang* reduces insulin resistance and also exhibits higher impact on progression delay of type 2 diabetes. Similar to *doubanjiang*, *tauchu* is also found to possess antidiabetic properties (Kwon *et al.,* 2010).

- **Miso**

Miso is a traditional fermented soy food that originated from Japan. *Aspergillus oryzae* strain is used during the fermentation process. Along with soy, salt, wheat, rice or oats is added to enhance the flavor of the fermented product. Thus, the product Miso is rich in vitamins, isoflavones, proteins, carbohydrates, minerals, and lecithin. Health benefits of this include prevention of liver, breast, and intestinal tumors, prevention of injury due to radiation, and prevention of hypertension (Watanabe, 2013).

- **Gochujang**

It is a traditional Korean food fermented with *Aspergillus* sp. and *Bacillus* sp. Soy bean is mixed with salt, red pepper powder, and rice powder and kept in the earthen pots for over a period of one year. It possesses antihypertensive activity, anticancer effect, and hepatoprotective properties which might be due to the presence of isoflavones (Kim and Lee, 2014).

- **Douchi**

Douchi is a popular salt fermented soybean food among Chinese food products. It is made from black soybeans. Microorganisms employed in the fermentation process are *Aspergillus, Mucor,* and *Rhizopus.* Douchi is used as a flavouring ingredient for a variety of dishes. The major health benefits include relieving tiredness, weakness, insomnia, and poor appetite. It also possesses antioxidant and α-glucosidase inhibitory activity (Iwaniak *et al.,* 2014).

4 Fermented Sea Food Products

Fermented sea food products are traditional foods in many countries. They are the source of many healthy molecules, but the only disadvantage is that they contain high amount of salt. Fermented sea food products are found to exhibit antimicrobial, antioxidant, antidepressors, immune modulatory, antihypertensive, anti coagulant and fibrinolytic activities.

- ***suan-yu***

suan-yu is a Chinese traditional low-salt fermented whole fish. It has high nutrition, unique flavor, and long storage capability. This fermented fish contains several strains of *L. plantarum.* These strains exhibit antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* via the production of bacteriocins (Zeng *et al.,* 2014).

- ***bekasam***

bekasam is an Indonesian fermented-fish product that tastes sour. Lactic acid bacteria isolated from *bekasam* are potent growth inhibitors of the pathogenic bacteria *E. coli, Salmonella*

FIGURE 23.7 Blue-mussel sauce.

typhimurium, *Bacillus cereus, Staphylococcus aureus*, and *L. monocytogenes*. This antimicrobial property is due to the presence of organic acids in these isolates (Martínez-Álvarez *et al.,* 2017).

- **Blue-mussel sauce**

Free radical scavenging activity was found in several traditional high salt fermented sea food products. During the fermentation process, a number of small peptides are released and these peptides are responsible for their high antioxidant potential (Figure 23.7). Some antioxidant peptides are isolated and characterized from traditional fermented sea food products. These peptides include the hepta-peptide sequence HFGNPFH with molecular weight 962 kDa and a peptide sequence FGHPY, with molecular weight 620 Dalton isolated from fermented blue-mussel sauce which are found to be potent free radical scavengers (Martínez-Álvarez *et al.,* 2017).

- **Health benefits of other fermented sea food products**

Many fermented sea food products act as sources of useful antihypertensive peptides. Angiotensin-converting enzyme (ACE) inhibition property has been found in many seafood sauces, including, anchovy, sardine, and salmon sauce. Some peptides with different molecular weights were isolated from fermented sea food sauces such as mussel and oyster-fermented sauces and are purified. They are found to exhibit ACE inhibition activity. Some fermented fish including *katsuobushi*, *rakfisk*, *heshiko* and *narezushi* were also reported for their ACE-inhibition capacity. A number of peptide sequences with strong and high ACE-inhibitory activity have been identified. Angiotensin II is a substance present in our body which normally narrows the blood vessels which results in high blood pressure and this can be prevented by Angiotensin-converting enzyme inhibitors thereby reducing the blood pressure. Thus, the dietary intake of these naturally occurring ACE inhibitors could act as a potent antihypertensive substance proving their beneficial effects (Martínez-Álvarez *et al.,* 2017).

Anticoagulant drugs are used for the management of heart diseases and stroke. Anticoagulant peptides are found to be present in some of the fermented sea food products. Salt-fermented anchovy sauce contains a stable fibrin-clotting inhibitor with molecular mass between 1 and 5 kDa. Another fermented sea food product, shrimp paste has shown strong fibrinolytic activity because of the presence of a novel fibrinolytic enzyme formed during fermentation. This enzyme will not cause any damage to the other blood proteins that are not involved in blood clotting cascade. High fibrinolytic activity has been found in fermented small cyprinid fish (*Puntius sophore*), a traditional fermented product from northeast India (Singh *et al.,* 2014).

5 Fermented Beverages

Fermented beverages have been consumed by humans for thousands of years. Health benefits of fermented beverages, including kombucha, wine and beer, are described further (Figure 23.8).

5.1 Kombucha Tea

Kombucha tea is a refreshing beverage fermented with kombucha culture (Figure 23.9). Since kombucha tea is a fermented product, it contains trace amounts of alcohol (0.5–2%). It contains significant quantities of vitamin B. Fermentation time and type of tea used during fermentation plays a major role in flavouring of this beverage, and sometimes fruit juices and herbs are added to the tea to enhance the flavor. It detoxifies blood, reduces cholesterol and blood pressure, and possesses anti-inflammatory, antidiabetic, and anti-atherosclerotic properties (Jayabalan *et al.,* 2016).

a) Antimicrobial activity

Kombucha inhibits the growth of several microbes such as *Aeromonas hydrophila, Agrobacterium tumefaciens, Bacillus cereus, Campylobacter jejuni, Candida albicans, Escherichia coli, Entamoeba cloacae, Helicobacter pylori, Pseudomonas aeruginosa, Salmonella choleraesuis serotype, Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermis, Vibrio parahaemolytica,* and *Yersinia enterocolitica*. Antimicrobial activity of kombucha is due to the presence of higher concentration of acetic acid, catechins, and unidentified proteins and compounds produced during fermentation (Jayabalan *et al.,* 2014).

b) Antioxidant Activity

Kombucha tea contains polyphenols and ascorbic acid that are responsible for its antioxidant activity. Because of the presence of these compounds, kombucha also possesses anticarinogenic activity (Hoon *et al.,* 2014).

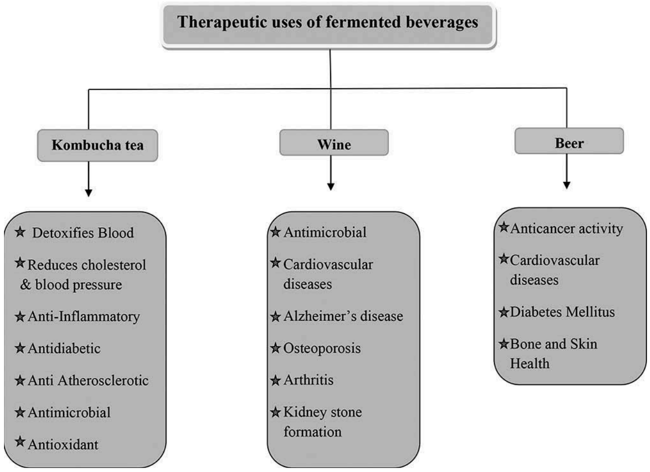

FIGURE 23.8 Therapeutic uses of fermented beverage.

FIGURE 23.9 Kombucha tea.

5.2 Wine

Wine is an alcoholic beverage made from grapes (Figure 23.10). For the production of red wine, grape peel is also added and on the other hand, for white wine production peel is not added. Yeast is used as a starter culture for the fermentation process. This yeast ferments sugar present in the grape juice to ethanol and $CO_{2.}$ Wines are subjected to aging before consumption, and based on the variety of wine, the period of aging differs. Moderate consumption of wine exerts health benefits which are discussed below.

FIGURE 23.10 Wine.

FIGURE 23.11 Beer.

a) Antimicrobial Activity

Wine exerts antimicrobial activity against pathogenic microorganisms such as *Campylobacter jejuni*, *Helicobacter pylori*, *Listeria monocytogenes*, *Salmonella* sp., *Staphylococcus aureus*, *Vibrio cholerae*, and *Streptococcus mutans*. The antimicrobial activity of wine is not only due to the presence of its alcoholic content, but it is also due to the presence of phenolic compounds, fatty acids and its acidic nature.

b) Other health benefits

The principal components in wine are flavonoids and stilbene and thus possess antioxidant activity. Wine reduces the risk of cardiovascular disease by enhancing the production of HDL, reduces osteoporosis, effective against Alzheimer's disease, reduces arthritis, decreases the incidence of kidney stone formation and reduces the risk of type 2 diabetes (Jackson, 2016).

5.3 Beer

Beer is produced from malted barley, water, yeast and various carbohydrate sources such as brewer's liquid glucose, liquid sucrose, unmalted barley, and cereal starches (Figure 23.11). It is a commonly consumed fermented beverage. Hops (a kind of flower) are added during beer production to enhance flavor and to give a bitter taste and also it favours the growth of *S. cerevisiae*, the major microorganism involved in beer production. It possesses some health benefits because of the presence of polyphenolic compounds. The major health benefits of beer include anticancer activity against breast, ovarian, pancreatic, prostate, colon and blood cancer, prevents cardiovascular disease and diabetes mellitus, and improves bone and skin health (Elrod, 2018).

6 Other Fermented Food Products

6.1 Vinegar

Vinegar is one of the oldest fermented products containing specific amounts of acetic acid and water used for human consumption. From centuries ago, it had a wide variety of industrial, medical, and house hold applications. Vinegars contain flavonoids, phenolic acids, and aldehydes,

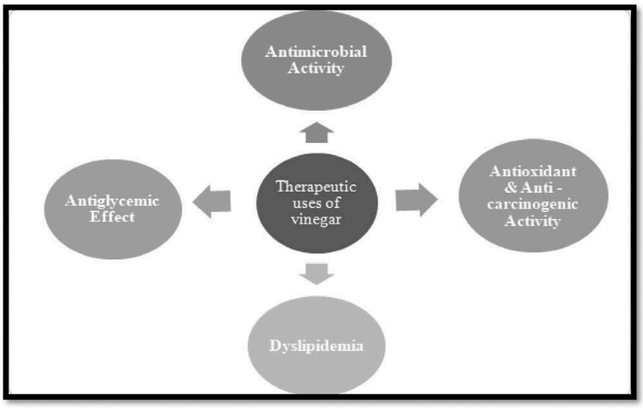
Antimicrobial Activity
Antiglycemic Effect
Therapeutic uses of vinegar
Antioxidant & Anti - carcinogenic Activity
Dyslipidemia

FIGURE 23.12 Therapeutic uses of vinegar.

depending on the source used for the production. Different types of vinegar such as wine vinegar, apple cider vinegar, Swaru salacca vinegar and Kurosu vinegar are found to exhibit the following therapeutic applications (Figure 23.12).

a) Antimicrobial Activity

Vinegar is commercially used as a preservative because of its antimicrobial properties (Budak *et al.,* 2014). Antimicrobial activity of vinegar is due to the presence of acetic acid. This acetic acid passes through bacterial cell membrane, thereby causing bacterial cell death. When compared to the other organic acids, acetic acid is found to be more effective against *Escherichia coli* even at lesser concentration (5% acetic acid) with very less exposure time (5 min) (Chang and Fang, 2007). Acetic acid (6%) is a powerful bactericidal agent against drug-resistant and multiple drug-resistant *M. tuberculosis* strains but the exposure time required is longer (30 min) (Cortesia *et al.,* 2014). Wine vinegar is a powerful antibacterial agent against *Campylobacter jejuni*, a major microorganism involved in causing food-borne diseases such as bloody diarrhea, dysentery, pain, and fever due to the consumption of chicken meat (Park *et al.,* 2014).

b) Antioxidant Activity

Oxidation is a process that produces free radicals and these free radicals may damage the cells of the organism. The compounds that lead to oxidation reaction are called oxidants and the compounds that inhibit oxidation are called antioxidants. Some of the common antioxidants are vitamin A, vitamin C, vitamin E, selenium and lutein. Due to the presence of phenolic compounds and melanoidins in vinegar, it exhibits potent antioxidant properties. Studies reveal that the antioxidant activity of wine vinegar is higher than apple cider vinegar but lower than persimmon vinegar (Verzelloni *et al.,* 2010).

c) Antiglycemic Effect

Before finding pharmacological glucose lowering therapy of vinegar, it was used as a home remedy for diabetes (O Keefe *et al.,* 2008). When vinegar is added to the meal, it reduces food intake because of feeling fullness, so glycemic effect of the meal is reduced (Johnston *et al.,* 2004). In recent studies, it is found that vinegar can be used for the treatment of diabetes (Gu *et al.,* 2012). But consumption of vinegar will not affect stomach histopathological structure, rather it protects pancreas from damage (Soltan and Shehata, 2012).

d) Dyslipidemia

Prolonged intake of acetic acid reduces serum cholesterol and triglyceride levels (Soltan and Shehata, 2012). Recently, it was found that the administration of Swaru salacca vinegar improved lipid profile which is evident from low LDL level (9.5 mg/dl), low TG level (58.25 mg/dl), high HDL level (60 mg/dl), and low total cholesterol (56.25 mg/dl) (Zubaidah *et al.,* 2017).

e) Anticarcinogenic Activity

An anticarcinogen is a substance that inhibits cell proliferation. The process of increase in cell number is called cell proliferation. In the case of cancer, there is an increase in cell proliferation. Kurosu vinegar inhibits the proliferation of many cancer cell lines including colon adenocarcinoma, lung carcinoma, breast adenocarcinoma, bladder carcinoma, and prostate carcinoma cells in a dose-dependent manner (Nanda *et al.,* 2004). Kibizu vinegar inhibits the growth and proliferation of human leukemia cells (Mimura *et al.,* 2004) and human squamous cell carcinoma cells by programmed cell death (Baba *et al.,* 2013).

Cereals are important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, in Asia, and in Africa. Fermentation causes changes in food quality indices including texture, flavor, appearance, nutrition, and safety. The advantage of fermentation may induce improvement in palatability and acceptability by developing improved flavors and textures; preservation through formation of acidulants, alcohol, and antibacterial compounds; enrichment of nutritive content by microbial synthesis of essential nutrients and improving digestibility of protein and carbohydrates; removal of antinutrients, natural toxicants, and mycotoxins; and decreased cooking times. The natural fermentation of cereals increases their relative nutritive value and available lysine. Bacterial fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids more than yeast fermentation, which mainly degrade carbohydrates.

7 Conclusion

Fermented food products contain more therapeutic values and are nutrient enriched. They are cost effective and some of these products can be made even at home. In this review, therapeutic values of some of the alcoholic fermented beverages are also included, but the limited consumption of these will give proposed health benefits but when the consumption exceeds, it may cause ill effects to the host. But there is a very less awareness among the public about the therapeutic values of fermented food products. Further research should be directed towards identifying the benefits and risks associated with specific indigenous fermented food products; elucidating the contributions of microorganisms, enzymes, and other constituents in the fermentation process; and developing starter cultures, unique microbial strains for nutritive improvement and detoxification, and testing of new varieties for their suitability as fermentation substrates may contribute for global food supply.

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